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DEEP-OCEAN BIODETERIORATION OF MATERIALS — PART III. THREE YEARS AT 5,300 FEET

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U. S. NAVAL CIVIL ENGINEERING LABORATORY
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DEEP-OCEAN BIODETERIORATION OF MATERIALS — PART III. THREE YEARS AT 5,300 FEET

Technical Report R-428

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by

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ABSTRACT

This is Part III of a series of reports on the biological deterioration of materials in the deep ocean. It covers the data obtained after exposing 1,318 test specimens of 316 different materials for 35 months on the Pacific Ocean floor at a depth of 5,300 feet (Test Site I). The materials were attached to a Submersible Test Unit (STU). The STU was retrieved in February 1965 and returned to the Laboratory for tests and analyses.

Hydroid growths were found on all the test specimens placed on the STU. A few species of tube worms were found attached to metals, plastics, and coated test specimens. Most of the plastics and all the rope materials were covered with bacterial slime growth. Cotton and Manila rope specimens were severely deteriorated by bacterial action. Wood panels, plastics, and Manila ropes were attacked by marine borers. Metals, natural and butyl rubber, and certain plastic materials were not affected.

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PREFACE

The U. S. Naval Civil Engineering Laboratory is conducting a research program to determine the effects of the deep-ocean environment on materials. This research will be of great value in establishing the best materials to be used in deep-ocean construction in the Navy's conquest of "inner space."

A Submersible Test Unit (STU) was designed, on which many test specimens can be mounted. The STU can be lowered to the ocean bottom and left for long periods of exposure. Planned exposures range from 4 to 48 months at depths from 2,500 to 18,000 feet.

Thus far, two deep-ocean test sites have been selected. Test Site I (nominal depth of 6,000 feet) is approximately 81 nautical miles southwest of Port Hueneme, California. Test Site II (nominal depth of 2,500 feet) is 75 nautical miles west of Port Hueneme. Additional test sites at depths of 12,000 and 18,000 feet may be chosen. This report deals with STU I-1, in place for 3 years at a depth of 5,300 feet.

INTRODUCTION

As part of a research program to determine the effects of the deep-ocean environment on various engineering materials, the U. S. Naval Civil Engineering Laboratory has placed six Submersible Test Units (STU's) on the ocean floor and five have been recovered. The first of the series, STU I-1, was emplaced at Test Site I in March 1962, followed by STU's I-2 and I-3 in October 1963 and STU I-4 in June 1964.

STU II-1 was emplaced at Test Site II in June 1964, followed by STU II-2 in April 1965. The location of the two test sites is shown in Figure 1.

STU I-3 was the first to be recovered, after 4 months of exposure at a depth of 5,640 feet. It was loaded with 1,367 test specimens of 492 materials. The effects of deep-ocean marine fouling and boring organisms upon these materials have been reported in Reference 1.

STU II-1 was the second STU to be recovered, after 6 months of exposure in 2,340 feet of water. It was loaded with 2,385 specimens of 603 materials. The effects of deep-ocean marine animals upon these materials have been reported in Reference 2. Preliminary results of corrosion of metals have been reported in Reference 3.

On 25 February 1965, STU I-1 loaded with 1,318 test specimens of 396 materials was the third STU to be recovered after 3 years (1,064 days) on the ocean floor in 5,300 feet of water (Figure 2). This report presents the materials and methods employed for attracting, collecting, and evaluating deep-ocean fouling and boring organisms, and the results of field and laboratory investigation of the recovered materials from STU I-1.

A fourth STU (STU I-4) was recovered on 18 July 1965 after 13 months exposure at a depth of 6,800 feet. The fifth STU was recovered on 22 October 1965 after 2 years exposure at a depth of 5,640 feet. The materials from both STU's are currently being evaluated and examined for corrosion and biodeterioration.

RESEARCH METHODS

Oceanographic Information

Concurrently with the STU program, numerous oceanographic and biological data-collecting cruises to the STU sites have been conducted.[4] These have produced information about the environmental parameters such as salinity, temperature, oxygen

content, and biological activity. This information is essential in evaluating changes in the materials exposed on the ocean floor, especially the corrosion of metals. The environment at both Test Site I and Test Site II is presented in Table I.

Test Site I was selected because the area provides a nominal 6,000-foot depth reasonably representative of the open-sea conditions in the eastern part of the Pacific Ocean. It is located approximately 80 nautical miles southwest of Port Hueneme, California.

Because the rate of corrosion of certain metals and alloys are greatly influenced by the amount of dissolved oxygen concentration in seawater, it was desired to investigate the effects of the minimum oxygen zone upon these materials. Test Site II was selected because at this site at a depth of about 2,500 feet, the dissolved oxygen content in seawater falls to a relatively low value and is known as "the minimum oxygen zone." Below and above this depth, the dissolved oxygen content increases. The underlying causes of the minimum oxygen zone are still imperfectly understood.

Biological Activity

When the Laboratory first decided to place STU I-1 on the ocean floor at Test Site I in March 1962, it was not known what kind of deep-sea creatures would be encountered, nor was the topography of the ocean bottom known. In order to obtain some information, a deep-sea camera was lowered from the stern of a vessel (YFU) while at the same time STU I-1 was being lowered to the ocean floor from the bow of the vessel. Numerous photographs were taken of the ocean floor close to where STU I-1 was placed.

The photographs revealed that the topography of the ocean floor in the vicinity of STU I-1 was generally flat except for numerous small mounds built by mud-dwelling animals. The presence of these mounds indicates that there is considerable biological activity at the test site. During the course of photographing the ocean floor, a 5- to 6-foot-long sharklike fish with a large dorsal fin came into view of the camera and was photographed apparently drifting near the ocean floor in 5,300 feet of water (Figure 3).

Rock Samples. Rock specimens were desired from this area to study fouling organisms attached to the rocks, since they could be expected to attach themselves to other materials placed there. A 10-inch-diameter by 36-inch-long steel pipe with retaining rods welded across the lower end of the pipe was employed for collecting rocks from the ocean floor. The pipe dredge was lowered to the ocean floor from an oceanographic vessel, USNS Davis, and the area dredged for rock specimens. Several passes were made across the area of Test Site I at a depth of 6,000 feet, and various-sized rocks were collected.

A variety of organisms were attached to the rocks (Figure 4). These are listed in Table II.

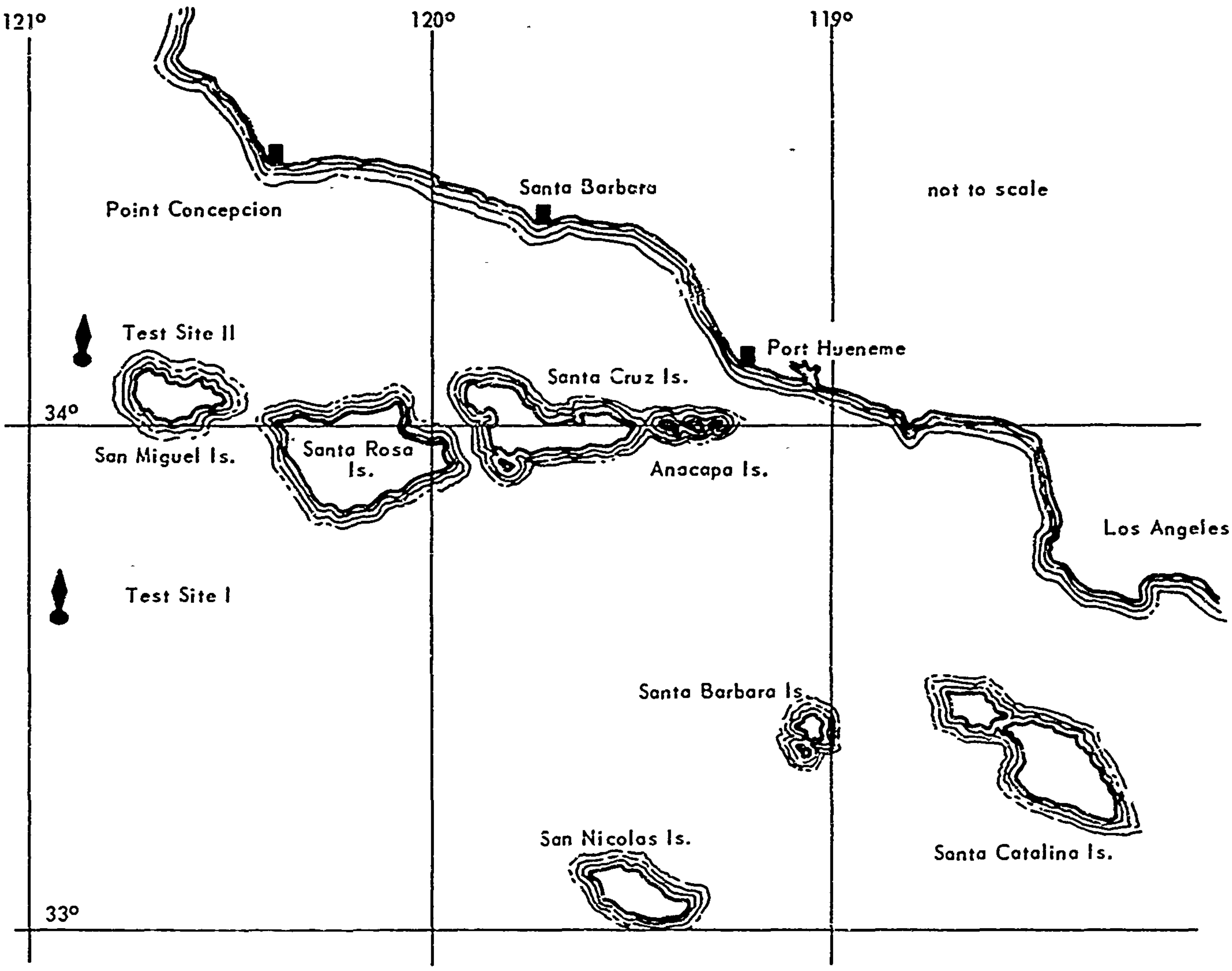


Figure 1. Test Sites.

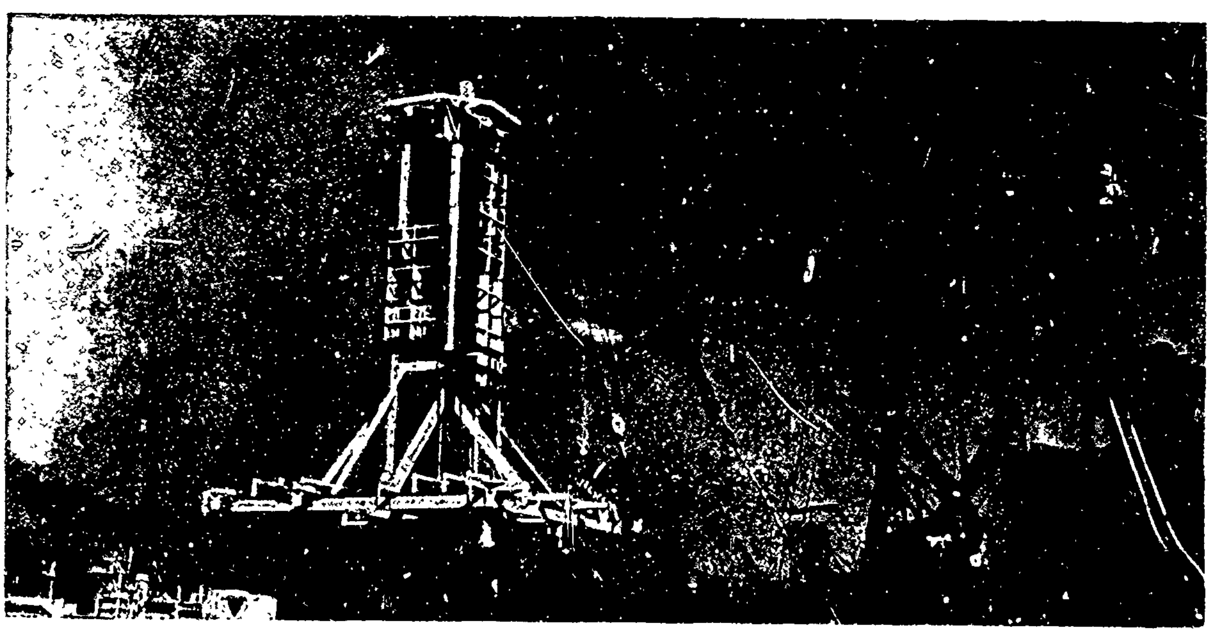


Figure 2. STU I-1 recovered from the sea being unloaded at dockside.

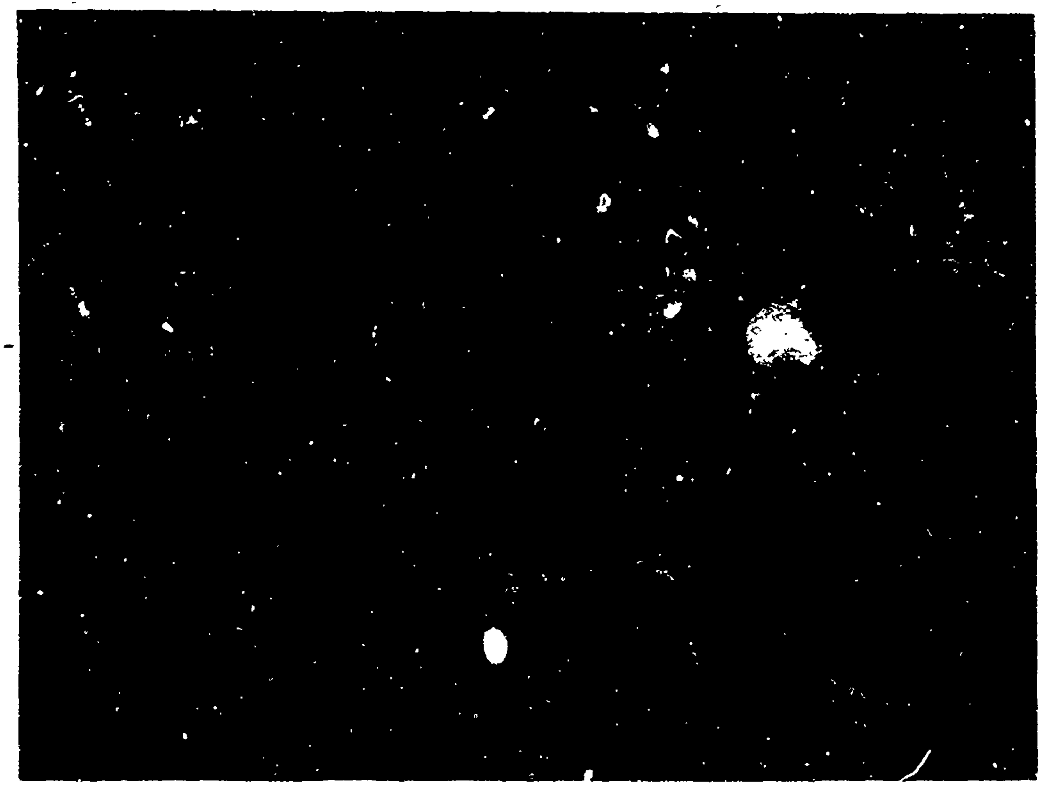


Figure 3. Sea floor photograph showing large unidentified fish (5 to 6 feet long). Note the large dorsal fin. Some luminescent organisms are present. (Courtesy of Carl Shipek of the U. S. Navy Electronics Laboratory.)

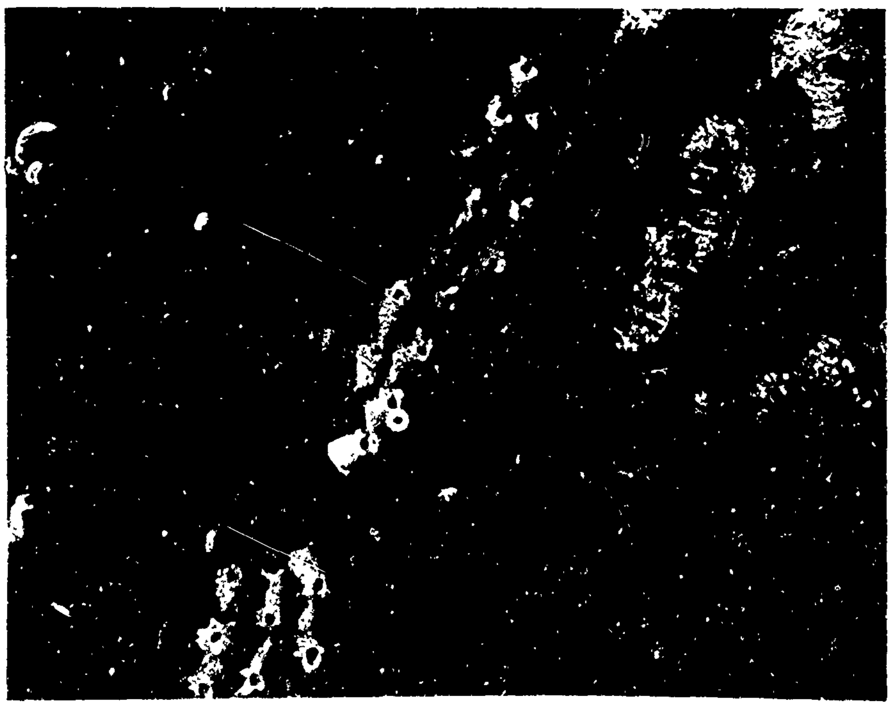


Figure 4. Photomicrograph of colonial (encrusting) bryozoa, deep-sea glass sponge, Foraminifera, and worm tubes on a rock surface (magnified).

Table I. Summary of Environments at Test Sites I and II

Factors	Surface Water	Test Site I	Test Site II
Depth, ft		5,300	2,340
Temperature, °C	13.0	2.53	7.2
Dissolved oxygen concentration, ml/L	5.6	1.26	0.42
Salinity, o/oo (ppt)	33.6	34.56	34.37
pH	7.9 - 8.0	7.44	7.46
Hydrostatic pressure, psi		2,500	1,030
Current, knots		Less than 0.5	0.3 max
Sediment		Green mud containing glauconite, Foraminifera, quartz, etc.	Green mud containing glauconite, Foraminifera, quartz, etc.

Table II. Fouling Organisms on Rocks From 6,000-Foot Depth

Organisms	Remarks
Bryozoa	Six or more different species of encrusting and erect forms; one encrusting form covered about 1 square inch of rock surface
Foraminifera	Many different species
Glass sponges	Several different deep-sea species
Serpulid worm tubes	Numerous large and small; the longest was over 4 inches
Brittle stars	One found in a pholad hole
Chiton	One 1/2 inch long

Several rocks had deep holes bored by pholads (Figure 5); neither live pholads nor their empty shells were found in the holes, so it is not known whether these rock borers are active at this depth. There was a thick deposit (as much as 1/4 inch) of manganese oxide on the rock specimens. There is a report of finding and isolating 36 pure bacterial species from inside surface-sterilized manganese nodules. It is thought that bacteria play a major role in the formation of nodules.[5]

Sediment Samples. Marine bacteria are one of the major biological agents in the deterioration and fouling of various materials submerged in the ocean. To determine the type and activity of bacteria in the deep ocean, sediment samples were obtained and analyzed in the laboratory using standard microbiological methods. Sediment samples were also obtained to determine the type of marine animals found in the sediment. The following samplers were used:

1. A gravity core sampler which takes cores up to 4 feet long.

2. NCEL's scoop-type sampler which collects about 225 cubic inches of sediment from a soft bottom.

3. A ZoBell bacteriological sampler which was modified in order to collect a mixture of seawater and sediment at the seawater-sediment interface. Samples were collected in a sterile unit and were not contaminated with bacteria from surface water during descent and ascent.

Figure 5. Rock sample from 6,000-foot depth showing numerous holes made by rock-boring animals. Bryozoa (erect type), tube worms, and legs of a brittle star are visible.

The samplers have been described in Reference 1. The results of the bacteriological analysis of the sediment samples are presented in Tables III, IV, and V.

The sulfate reducers found in the sediment samples are anaerobic bacteria which obtain their energy by the reduction of sulfate and sulfite in water in the absence of free oxygen. The end product of their metabolic process is hydrogen sulfide (H_2S). These microbes are considered to be responsible for the anaerobic corrosion of metals.[6] Microorganisms other than sulfate reducers, are also found to be responsible for metal corrosion.[7,8,9]

The number of aerobic, anaerobic, and sulfate-reducing marine bacteria was determined in the laboratory on nutrient agar plates and in test tubes. Because of the different nutritional requirements of marine bacteria and the limitation in the enumeration procedure employed in this study, only a small percentage of the bacterial population may have been demonstrated by the analysis of the bottom samples. The following media were used for culturing marine microorganisms.

Table III. Aerobic and Anaerobic Bacteria in Core Samples of Sediment

Sample No.	Depth (ft)	Depth of Sample Below Soil Surface (in.)	Aerobes (per gram of wet sediment)	Anaerobes (per gram of wet sediment)	Sulfate Reducers Present
C162	5,300	0 - 1	12,000	600	yes
		3 - 3.5	10	200	yes
		6 - 6.5	0	600	yes
		12 - 12.5	0	200	no
		24 - 24.5	0	50	yes
		36 - 36.5	0	0	no
C163	5,300	0 - 1	6,000	5,000	yes
C262	5,640	0 - 1	2,000	300	yes
		1 - 1.5	1,000	500	yes
		2.5 - 3.0	30	30	yes
C362	5,640	0 - 1	8,000	5,000	yes
		3 - 3.5	60	60	yes
		6 - 6.5	0	0	yes
		12 - 12.5	0	0	yes
		18 - 18.5	0	0	yes
C264	2,500	0 - 1	5,000	1,000	yes
		3 - 3.5	10	100	no
		6 - 6.5	0	0	no
		12 - 12.5	0	0	no
C164	2,500	0 - 1	6,000		yes
		3 - 3.5	10		yes
		6 - 6.5	0		no

Table IV. Aerobic Bacteria in Scoop Samples of Sediment[1]

Sample No.	Depth (ft)	Aerobes (per gram of wet sediment)
M662	5,300	25,000
M163	5,640	30,000
M264	2,500	10,000

1/ Sulfate-reducing bacteria present in all these samples

Table V. Aerobic and Anaerobic Bacteria in Sediment Obtained With a ZoBell Bacteriological Sampler[1]

Sample No.	Depth (ft)	Aerobes (per gram of wet sediment)	Anaerobes (per gram of wet sediment)
Z462a	5,300	300,000	not tested
Z462b	5,300	500,000	not tested
Z5a62	5,300	1,500,000	500,000
Z5b62	5,300	1,870,000	500,000
Z5d62	5,300	1,700,000	600,000
Z11a63	5,640	1,500,000	600,000
Z11b63	5,640	2,000,000	600,000

1/ Sulfate-reducing bacteria present in all these samples

The nutrient medium for determining the general aerobic bacterial population consisted of:[10]

Bacto-peptone	5.0 gm
Ferric phosphate	0.1 gm
Yeast extract	1.0 gm
Seawater (aged)	1,000 ml
Bacto-agar	20 gm
pH	7.5

To determine the general anaerobic bacterial population, the above medium was then treated with sodium formaldehyde sulfoxylate (1.0 gm/l) to lower the redox potential, and with resazurin (0.001 gm/l) to serve as an indicator of anaerobiosis.[10]

The nutrient medium for determining the presence of sulfate-reducing bacteria consisted of:[10]

Potassium phosphate dibasic	0.2 gm
Magnesium sulfate hydrated	0.2 gm
Sodium sulfite	0.1 gm
Ferrous ammonium sulfate	0.1 gm
Calcium lactate	3.5 gm
Ascorbic acid	0.1 gm
Bacto-peptone	1.0 gm
Yeast extract	1.0 gm
Bacto-agar	3.0 gm
Seawater	1,000 ml

The pH was adjusted to 7.5, and the medium (agar excluded) was placed in test tubes with screw caps and sterilized. The screw caps were tightened when the medium was still warm to exclude atmospheric oxygen. Small samples of bottom sediment from various depths were placed in these test tubes and incubated for several days at 15°C.

The sediment samples obtained with a scoop sampler were washed through a plastic screen to collect mud-dwelling organisms. The animals were bottled and preserved in a 5-percent glycerol - alcohol solution aboard ship for laboratory analysis. A variety of animals were found in these sediment samples. Annelid worms were the most abundant marine organisms collected in the vicinity of STU I-1 (Figure 6). Other mud dwellers collected were nemertean or round worms, holothurian or sea cucumbers, (Figure 7), molluscans, and Foraminifera tests. The Foraminifera tests found in the sediment samples have been classified and reported in Reference 1.

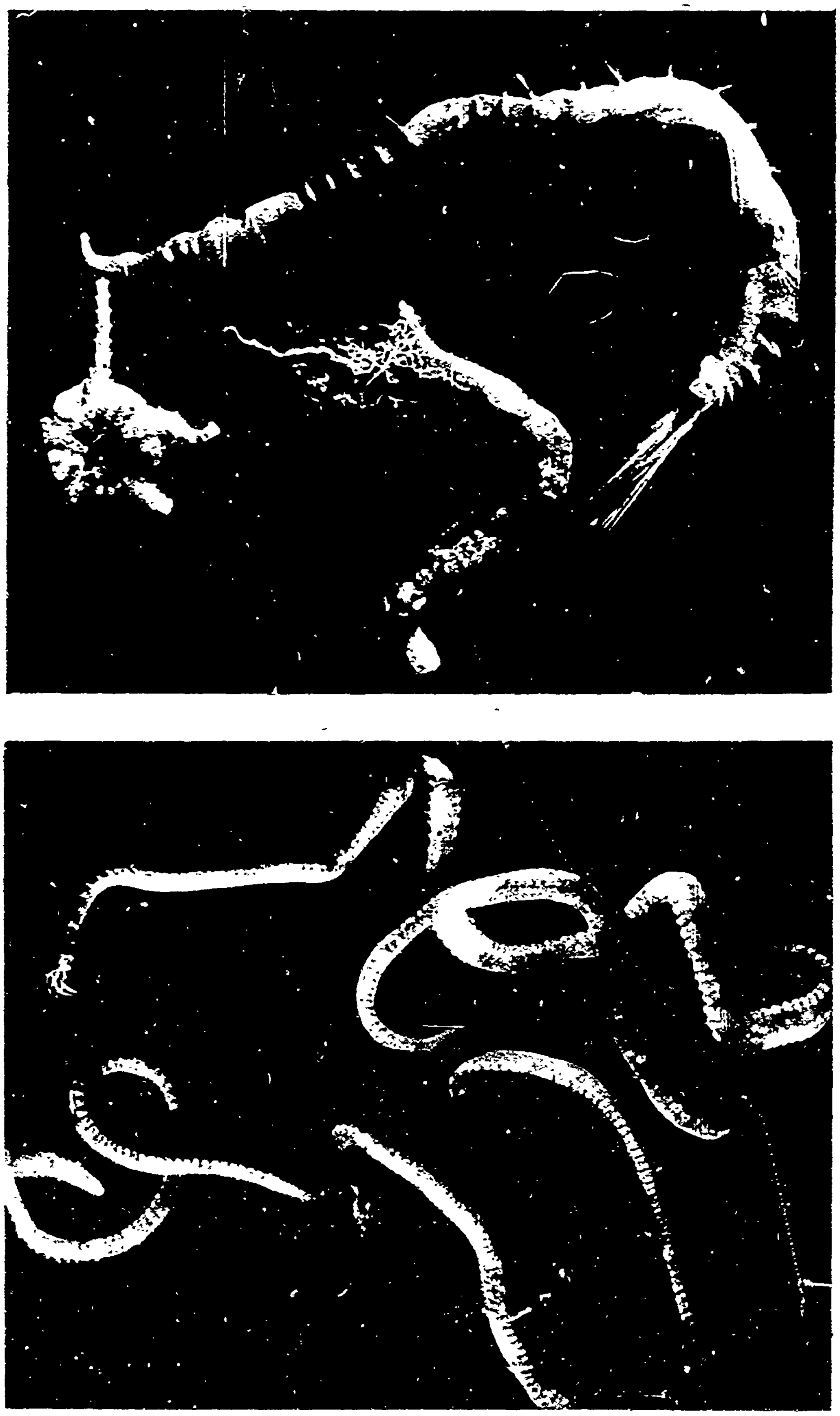


Figure 6. Annelid worms up to 6 inches in length found in a sediment sample.

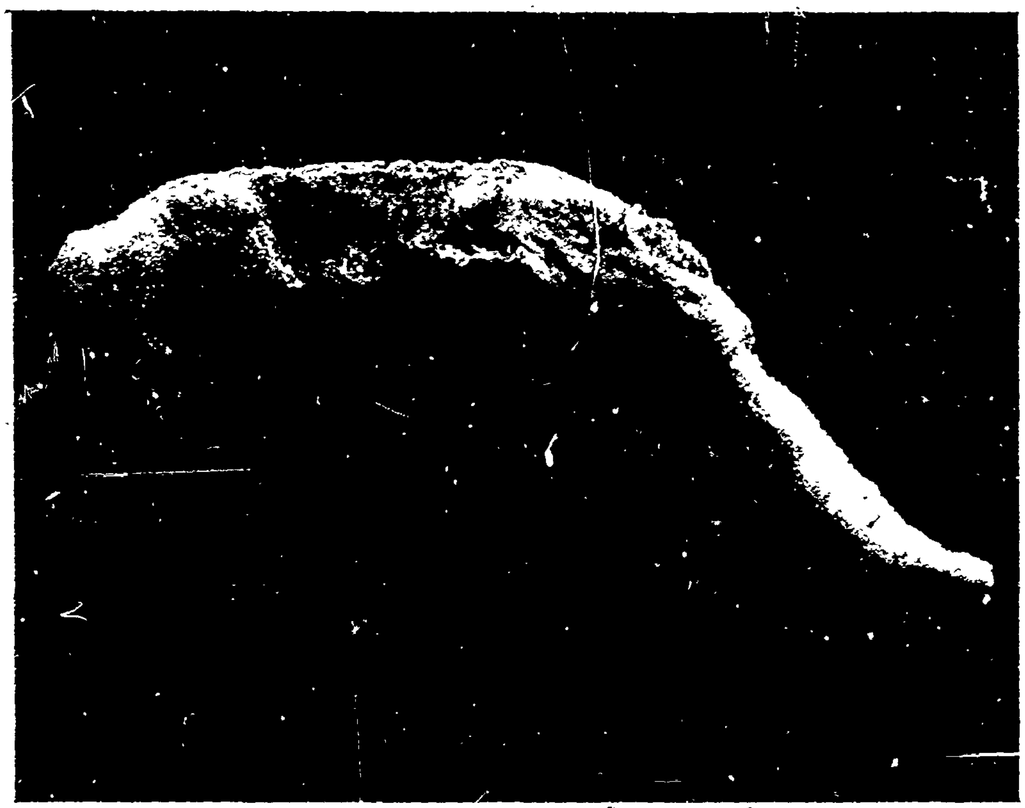


Figure 7. A 1-inch-long holothurian (sea cucumber) found in a sediment sample.

Antibiotic-Producing Marine Bacteria

A bacteriological analysis was made of a sediment sample obtained from a depth of 1,500 feet of water off the coast of Port Hueneme during the early phase of this study. On one of the Petri dishes (pour plate) inoculated with the sediment sample and incubated for several days at 15°C, a relatively clear area surrounding one of the bacterial colonies was observed. The orange-pigmented bacteria had apparently produced an antimicrobial agent known as an antibiotic which inhibited the growth of other microorganisms. It plays an important role in regulating the microbial population of bottom sediment. The 3/16-inch-diameter colony had produced a 5/16-inch-wide clear area surrounding the colony. A few small yellow-pigmented bacterial colonies were not affected by the antibiotic substance and were growing inside the clear area.

The nutrient medium used on which the bacteria produced the antibiotic substance had the following composition:

Bacto-tryptose	4 gm
Bacto-neopeptone	1 gm
Yeast extract	0.5 gm
Ferric phosphate	0.1 gm
Bacto-agar	15 gm
Seawater (aged)	1,000 ml
pH adjusted to	7.2 - 7.5

The bacteria had the following morphological and cultural characteristics:

Cell:	Gram negative rod
Agar colony:	Orange-pigmented, circular, and flat
Growth in nutrient medium made with distilled water:	Trace of growth
Growth in nutrient medium made with seawater:	Excellent growth

This indicates that the bacterium was a true marine specie, and not a transplanted terrestrial specie.

Test Materials

For evaluating deep-ocean biological effects on nonmetallic specimens, two racks (bio-racks) were attached to the STU. The bio-racks were made of 1/4-inch-thick mild steel, and were covered with a vinyl red lead primer paint. The racks held ten plastic rods, four tubes, one pipe, and one rubber tube, all 3 feet long (Figure 8).

The sections of the 3-foot-long plastic rods, tubes, and pipe, and rubber tube were treated in different ways. One section of each specimen was roughened, a second section was wrapped in burlap (a coal tar was used to adhere the first layer of the burlap to the specimen), a third section was taped with a friction tape and plastic electrical tape, and the fourth section was left smooth. The various wrappings were to provide a favorable foothold for the attachment and growth of deep-ocean fouling and boring organisms. A large piece of untreated fir wood was fitted around each of the specimens to act as bait to attract and lead any marine boring animals into direct contact with the specimen materials.

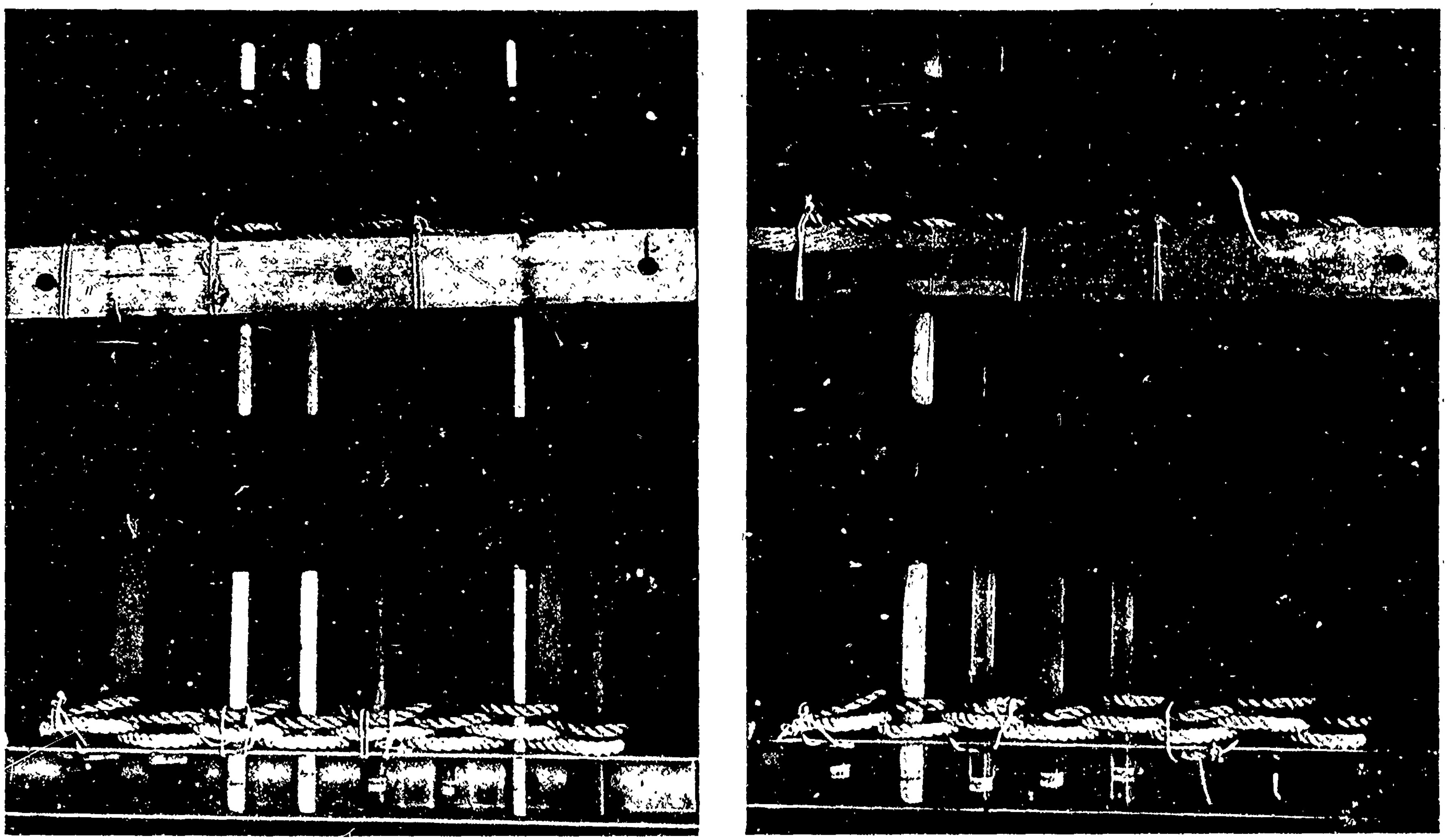


Figure 8. Various nonmetallic test materials assembled in biological racks before exposure.

Natural fiber ropes such as cotton and Manila were placed on the bio-racks. One set of ropes was placed at the lower and another set at the upper section of the bio-racks. The ropes were held in place by interweaving them through the plastic specimens, and then tying them to the plastic specimens with a nylon parachute shroud line as shown in Figure 8. Other materials such as electrical conductors covered with 0.015-inch-thick insulation over a No. 16 tin-coated copper wire, and plastic sheet materials were secured to the bio-racks.

In order to expose the test materials to biological deterioration in sediment (where bacteria are found to be most active) as well as in water, the two bio-racks were attached to the STU frame so that the lower portion would be buried in the bottom sediment and the upper portion exposed to seawater about 3 feet above the mud line (Figure 9).

Other materials placed on the STU for the biodeterioration study include single and multiconductor electrical cable materials (Figure 10), and 3 x 6.2-inch cylindrical concrete specimens (Figure 11). Coral concrete cylinders were selected for exposure because mollusks including Lithophaga, Gastrochaena, and Petricola are known to bore into dead coral or coral limestones. Living coral colonies are not attacked by the burrowing animals to the same extent as dead coral. There are waterfront as well as nonwaterfront structures made of coral concrete on the island of Guam. The list of materials placed on STU I-1 for the biological deterioration study is presented in the Appendix. Additional information about these materials are given in the Appendix.

Materials containing antifouling paints or other toxic substances were excluded from exposure aboard the STU. The current velocity at a depth of 5,300 feet was not great enough (less than 0.5 knot) to carry away any toxic substance which might alter the natural biological fauna found in the immediate vicinity of the STU.

RESULTS

Marine Growth on STU Complex

A report on the method used to emplace STU I-1 complex on the ocean floor in 5,300 feet of water is presented in Reference 11. The sketch of the complex is shown in Figure 12. The subsurface gasoline-filled rubber buoy, the instrument package attached to the riser line, and the 1-inch-diameter twisted polypropylene riser line were lost sometime during the 35-month exposure period.

The STU was retrieved by a grappling operation from aboard the USS Chickasaw, a fleet tug. The grappling hook snagged the nylon line and the chain which were stretched across the ocean floor and connected to the upper section of the STU frame.

The recovered section of the polypropylene rope used as an inverted catenary line had a dense hydroid growth. Some of the hydroids were about 5 inches long. Slimy bacterial growth, annelid worms, white starfish, and actinarians (sea-anemones)

were also found attached to the rope (Figure 13). The recovered polypropylene and nylon ropes were examined for signs of lacerations or cuts by fish, but no evidence of such an attack was found. The seriousness of fish bites on plastic ropes exposed in the Atlantic Ocean has been reported.[12,13] Slight amounts of hydroid growth were found on the STU frame, which was coated with a white vinyl paint. Typical fouling growth such as barnacles, bryozoa, and other attachment organisms were not found on the painted surface.

Marine Growth on STU Materials

Metal Specimens. The majority of test specimens on the STU were metals and metal alloys to study the effects of the deep-ocean environment on corrosion.

The major fouling organisms found attached to the surface of metal specimens were species of hydroids belonging to the phylum Coelentra (the polyps). Trace to heavy hydroid growth were found on all the metal test specimens (Figure 14). Some of the hydroids were 3 to 4 inches long. The heaviest growth occurred on the surface of a 1 x 6 x 3/16-inch No. 316 stainless steel test specimen. Trace to very light growth occurred on the surface of copper based alloys and manganese bronze test specimens. The surface of the metal specimens where the hydroids were attached was not affected. This area was examined under a microscope for signs of corrosion in the form of pits.

When a 2 x 2 x 1/8-inch stainless steel coupon used as a spacer between a test-specimen holder and a wood panel was pried loose from a wood surface, there were four elongated pits on the surface of the coupon. The pits contained black corrosion products. Several marine borers had penetrated the wood and were in direct contact with the metal, but no pitting of the metal surface was evident; however, there were red rust stains at these sites.

Two different kinds of surpulid worm tubes constructed of different materials were found on the test panels. One of these tubes was made of white calcareous material which was hard and brittle. Several such tubes were found attached to the surface of stainless steel panels which were partly exposed in the sediment (Figure 15). Approximately 1/2 inch of one end of a 1-inch worm tube was extended outward at right angle to the panel. This particular form of tube construction made by a surpulid worm was also found on coated and painted test panels, and on plastic materials (Figure 16).

The second type of worm tubes was composed of fine and coarse sands, Foraminifera tests, glauconite, and debris cemented together. A tube approximately 4 inches long was found on the surface of a Hastelloy C metal panel which was partly buried in the sediment (Figure 17). After the panel was cleaned in an acid bath (18 percent HCl by volume), the impression of the worm tube was still clearly visible on the metal surface (Figure 18).

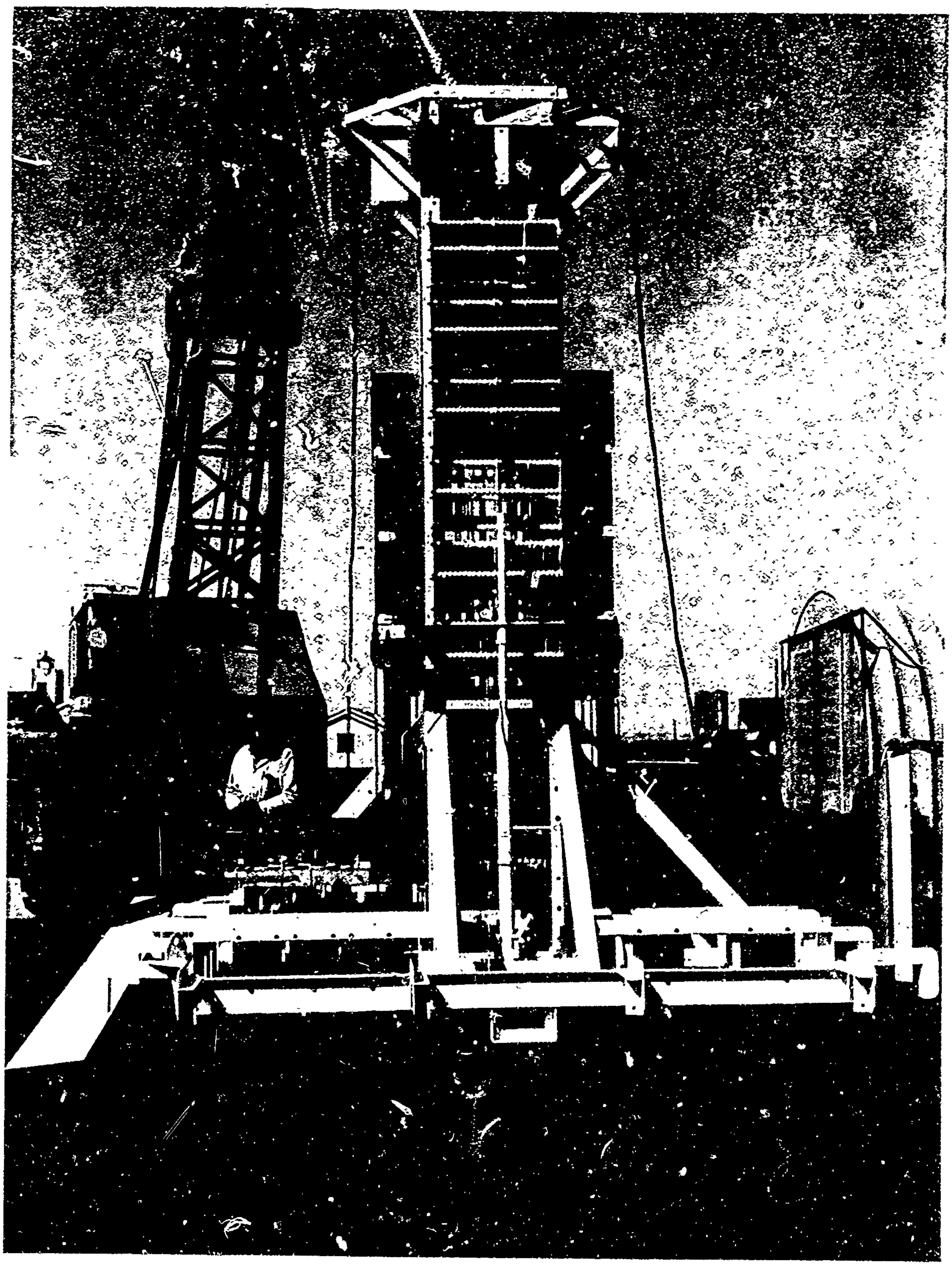


Figure 9. Biological test specimen racks secured to the frames of a STU before deep-sea exposure.

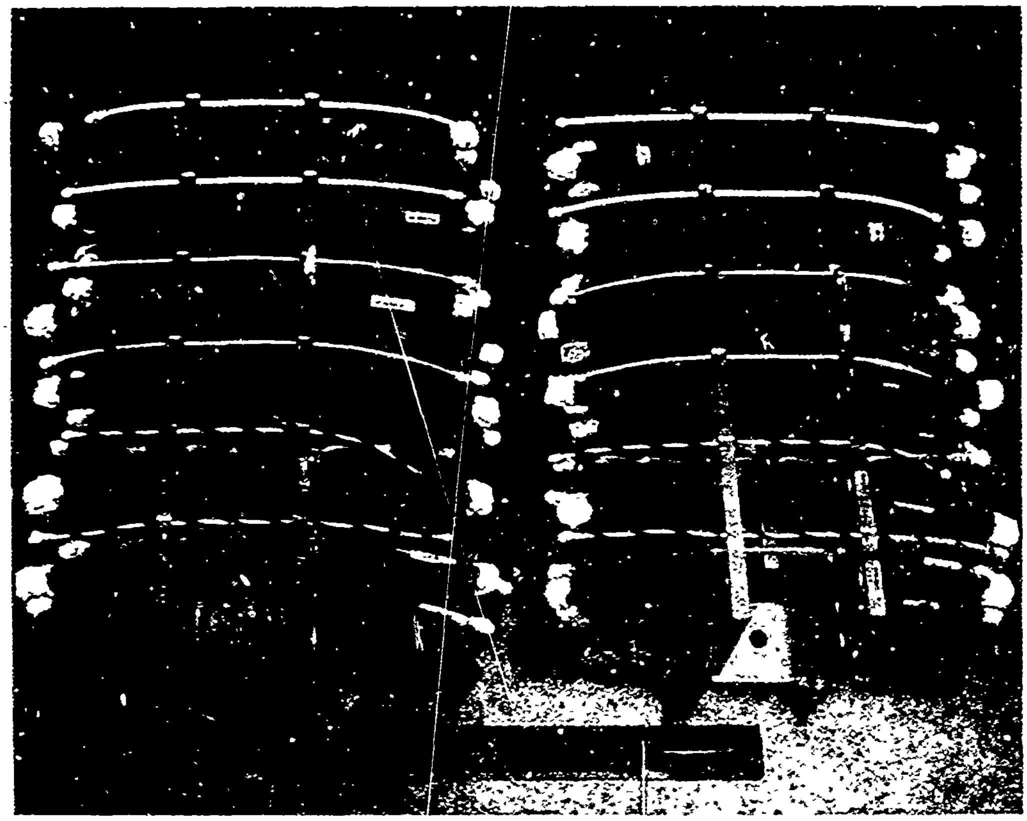


Figure 10. Single and multiconductor electrical cables before exposure. The ends are sealed with silicone rubber cement.

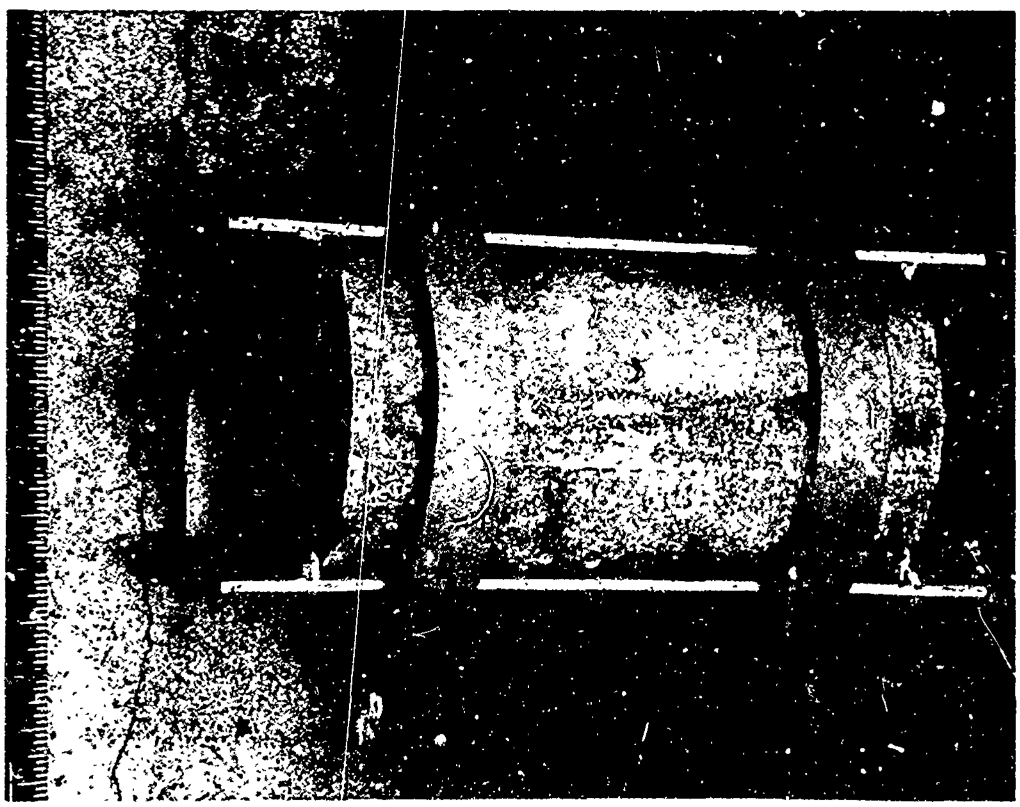


Figure 11. Concrete cylinder in a rack before exposure.

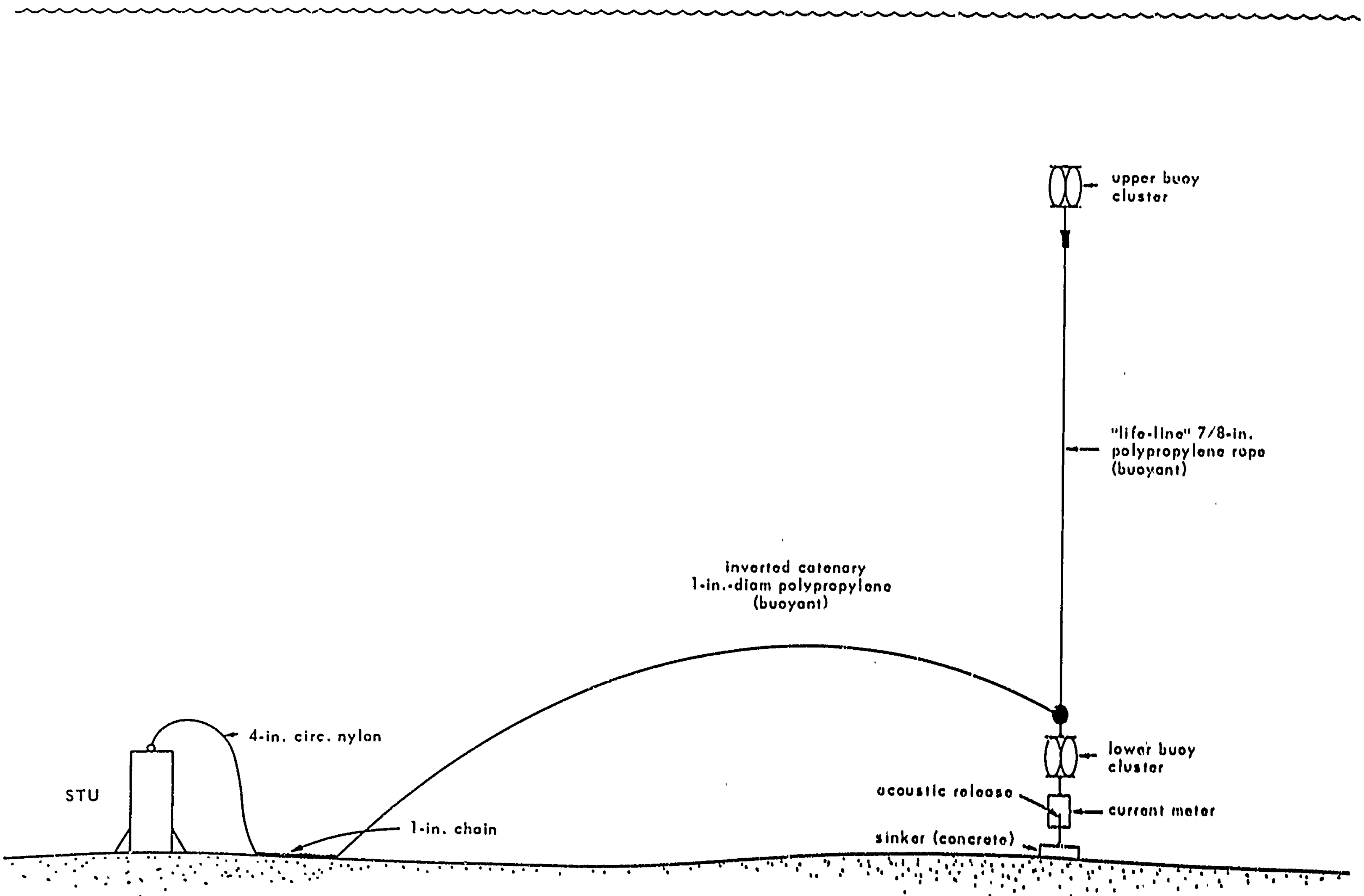


Figure 12. Schematic of STU complex.

Figure 13. Specimens of deep-sea annelid worms, white starfish, and sea anemones found attached to plastic rope.

Figure 14. Hydroid growth on metal test panels.

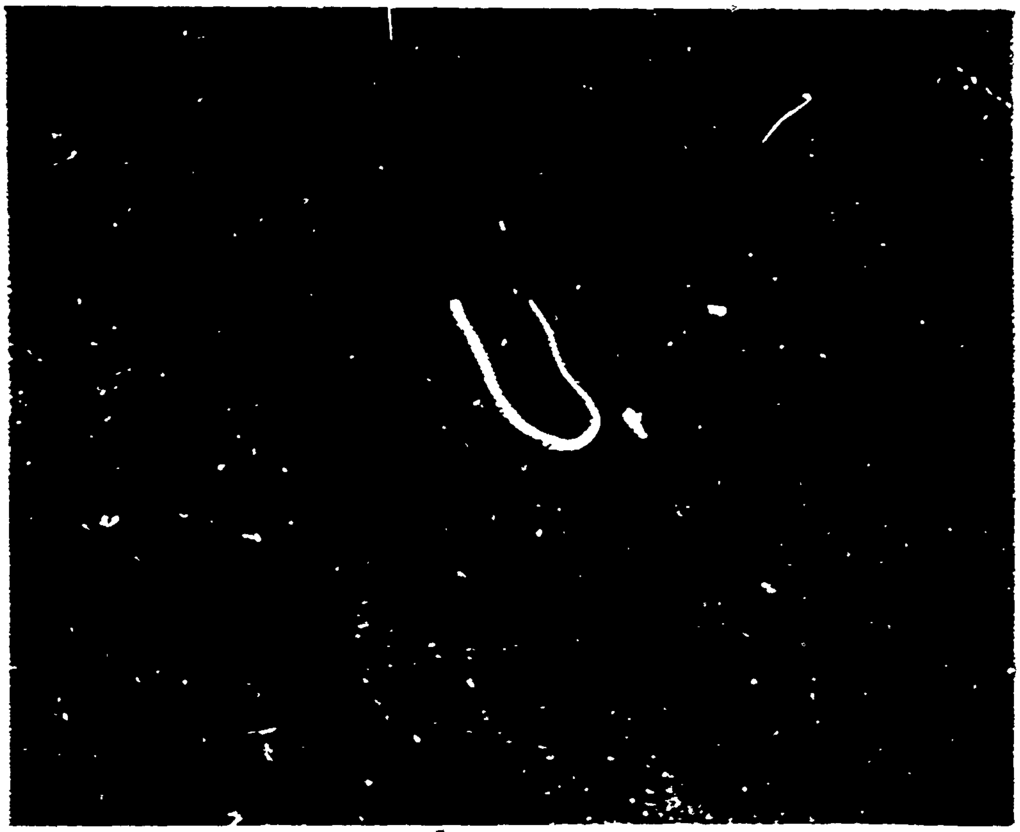


Figure 15. White calcareous surpulid tube worm attached to a stainless steel panel.

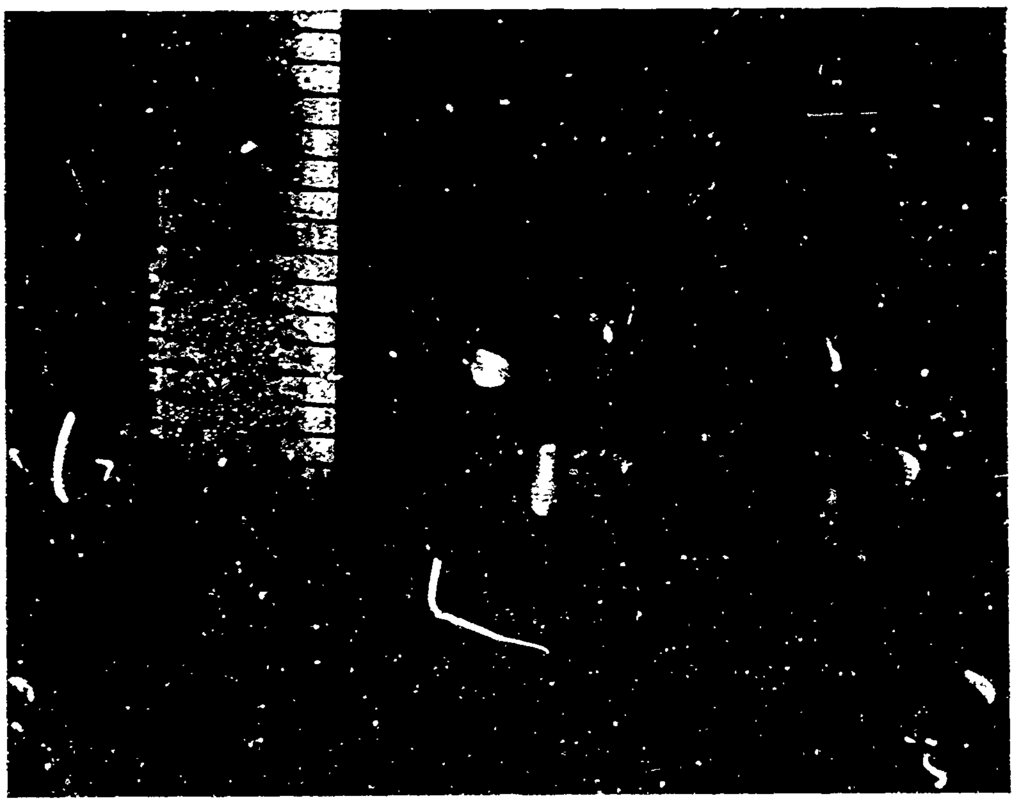
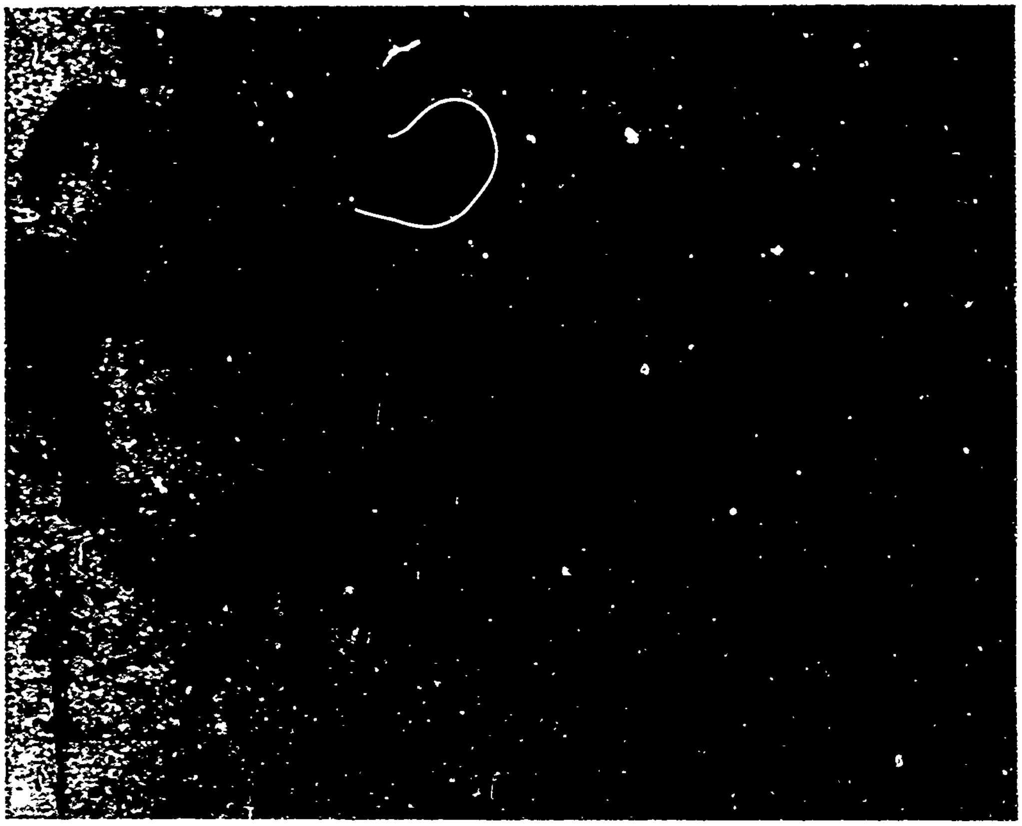


Figure 16. White calcareous surpulid tube worms attached to the surface of an acrylic plastic.

Figure 17. Surpulid tube worm on the surface of a Hastelloy C test panel.



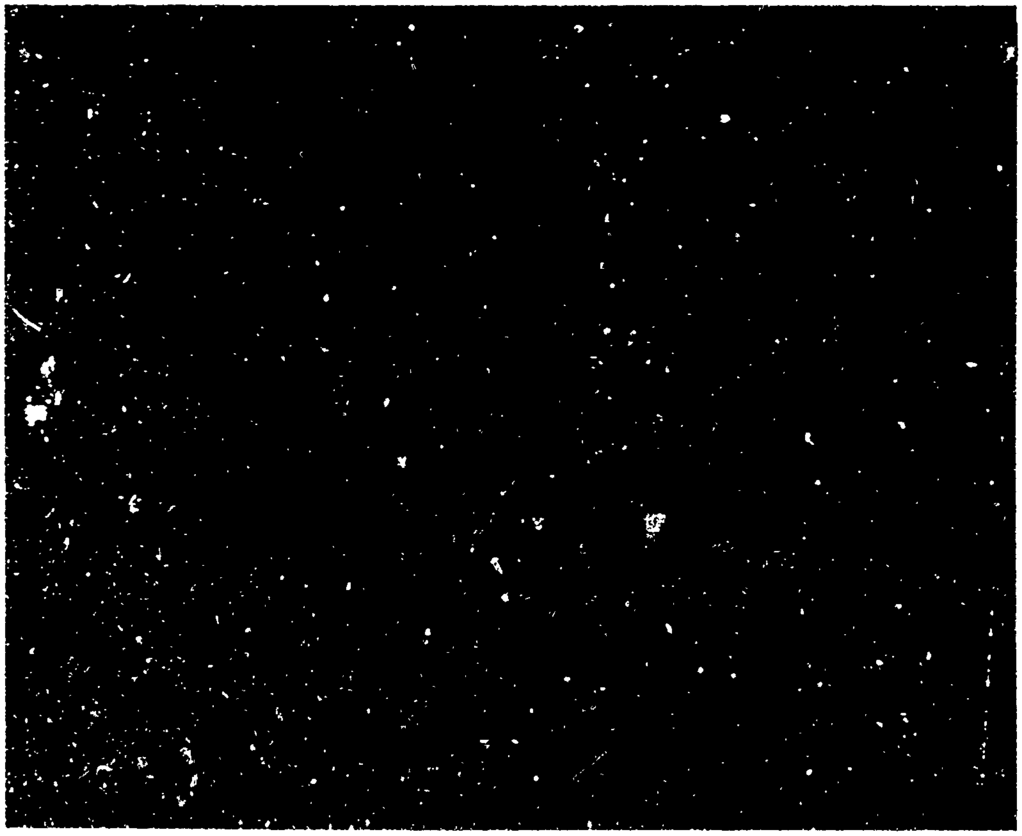


Figure 18. Impression of the surpulid tube worms and hydroid growth are visible on metal panels even after being cleaned in an acid bath.

Numerous stalked marine animals up to 2-1/2 inches long were attached perpendicularly to the surface of metal specimens. These organisms are also a specie of hydroid (Figure 19).

Fouling organisms such as barnacles and bryozoa were not found on any of the test specimens. Barnacles were of special concern because pits could result from localized oxygen-concentration cells formed on the surface of the metal beneath barnacle growth.[14] There are reports of finding barnacle species in the deep ocean.[15]

Nonmetal Specimens. Hydroid growth was found on all the nonmetal test specimens such as painted and coated panels and plastic and elastomer materials.

Numerous calcareous tube worms were found on the painted and coated panels, and on an acrylic sheet which was exposed about 8 feet above the sediment (Figure 16). A spiraled white calcareous worm tube was attached to the vinyl-painted metal test specimen holder about 6 feet above the bottom sediment (Figure 20).

A worm tube about 4 inches long which was made of fine sands and other materials was found attached to a painted test panel exposed about 5 feet above the bottom sediment (Figure 21). The materials used in the fabrication of this tube were of much smaller composition compared to the materials used to form the tube in Figure 17.

Marine Growth on Biological Test Materials

As soon as the STU was placed aboard the ship, it was examined for the presence of marine animals which were not attached to any of the test panels. The animals were collected and preserved in a 5-percent glycerol - alcohol solution for classification in the laboratory. One was a white lobsterlike crustacean with pinchers (Figure 22). The specimen was sent to the Smithsonian Institution for classification. It was identified as a galatheid lobster, Munidopsis verrilli Benedict (family Galatheidae). The animals found in the deep-ocean environment are normally dark colored, and finding a white crustacean on the ocean floor in 5,300 feet of water is very unusual.

The test panels secured to the lower portion of the STU were buried in the sediment as planned because samples of bottom mud were found adhered to the surface of a number of these test panels.

When the vinyl-painted biological test specimen holders were examined in the laboratory, it was found that the lower section of the "U" channel iron exposed near the sediment, expecially the area in contact with the rope specimens, had turned black (Figure 23). This was probably the result of hydrogen sulfide produced by the sulfate-reducing bacteria combining with the lead in the paint to form lead sulfide, which is black. The area under the decaying rope specimen where slime bacteria were active could have produced an anaerobic environment suitable for the growth of sulfate-reducing microorganisms. Except for the change in color, neither the paint nor the metal underneath the painted surface was damaged. Species of pectens and limpets were found on the painted surface exposed near the sediment (Figure 24).

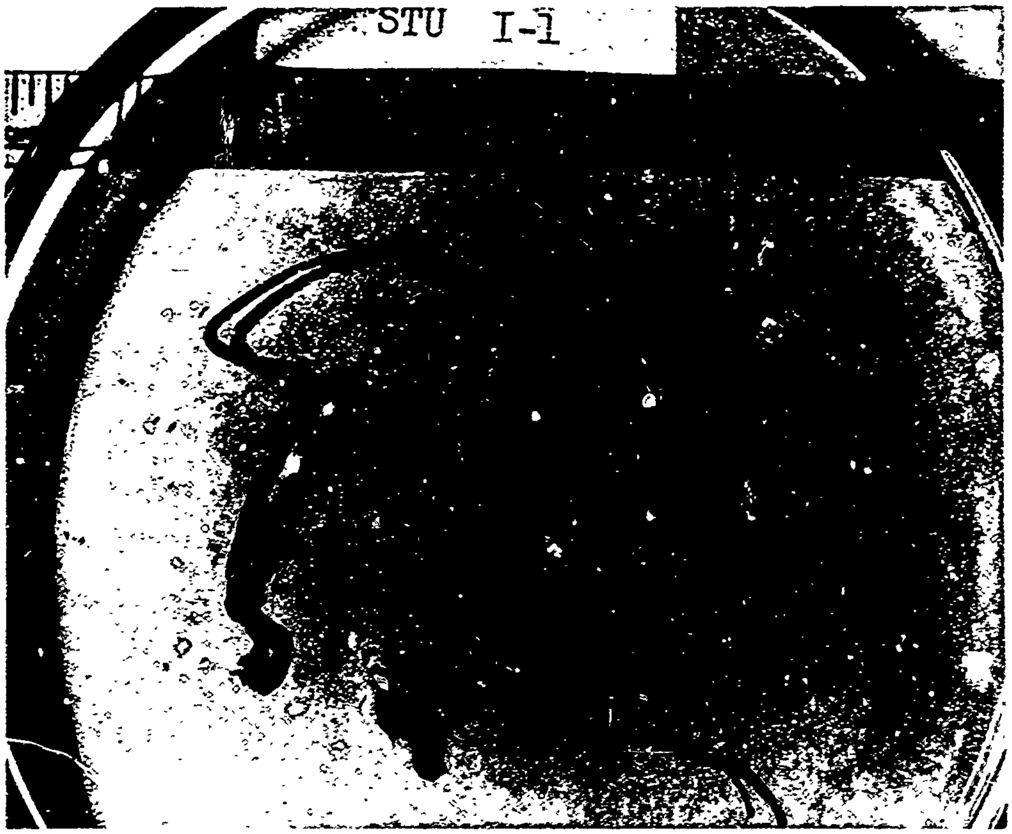


Figure 19. Stalked marine animal (hydroids) up to 2-1/2 inches long attached perpendicularly to the metal and plastic panel.

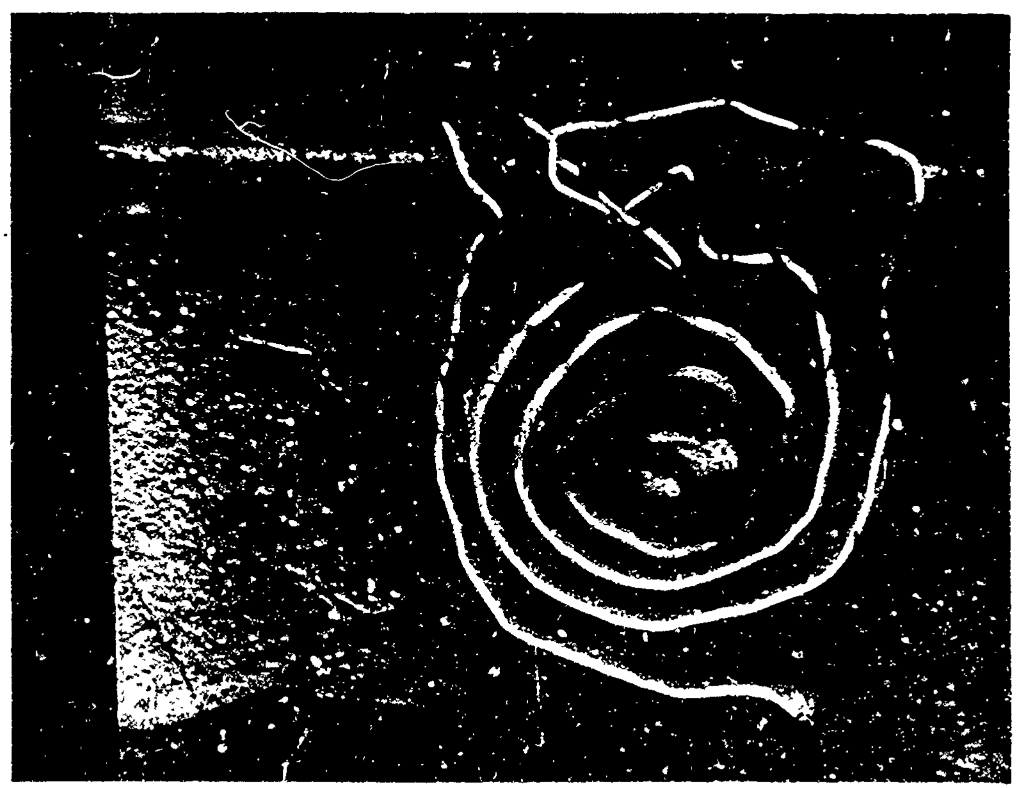


Figure 20. Spiraled white calcareous worm tube attached to the vinyl-painted metal specimen holder.

Figure 21. Surpulid tube worm on a painted test panel which was exposed about 5 feet above the sediment.

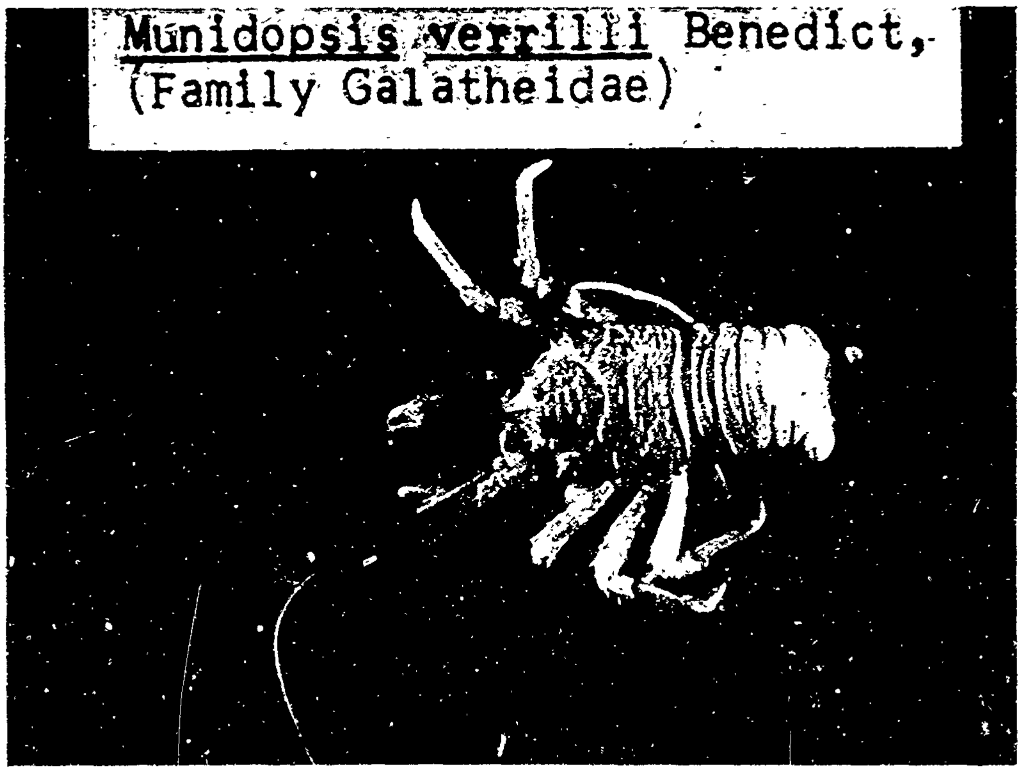


Figure 22. A white lobsterlike crustacean found on the STU.

Figure 23. Black area where the rope specimens were in contact with the orange painted surface.

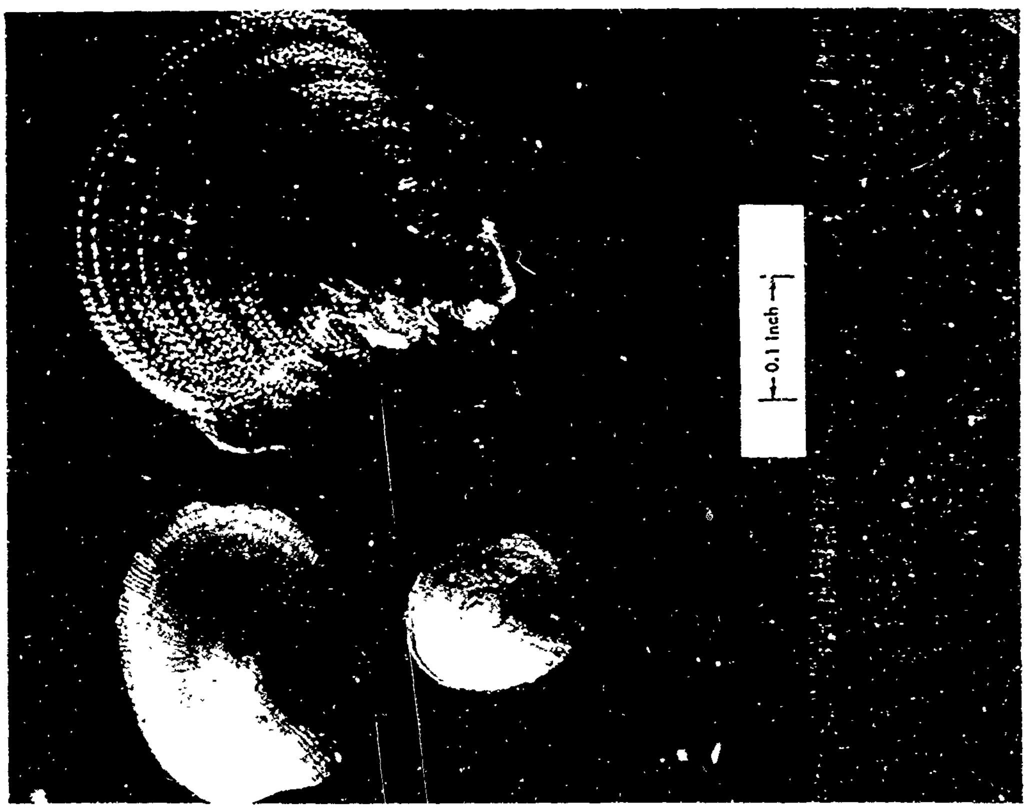


Figure 24. Species of deep-sea pecten and limpets found attached to STU materials.

The results and comments on the effects of deep-ocean organisms on various nonmetallic test specimens which were assembled on bio-racks (Figure 25) are presented in the Appendix. Close visual inspection of the recovered materials was performed under a stereoscopic microscope; insulation resistance and voltage breakdown tests were conducted on electrical conductors; and compressive strength tests were performed on cylindrical coral concrete specimens. A breaking-strength test on rope specimens could not be conducted since these materials were completely deteriorated by marine organisms. (Note dangling rope specimens in Figure 25.)

DISCUSSION

From the results obtained on the biological deterioration of materials exposed on the ocean floor at a depth of 5,300 feet for a period of 35 months, it is concluded that nonmetallic materials such as pine and greenheart wood, Manila and cotton ropes, silicone rubber insulation, friction tape, and jute fibers including burlap coated with coal tar are susceptible to total biological destruction and are not suitable for deep-ocean use.

All of the plastic rod and tube specimens were deteriorated in various degrees by marine boring animals in an area where a wooden bait piece was fitted around each specimen; however, other areas of the plastic materials were not affected. Some of the plastic materials such as cellulose acetate absorbed a considerable amount of water as compared to other plastic rods (moisture analyses were not performed). A vinyl plastic tube (NCEL No. 15) lost its plasticizer or some other chemicals used in the formulation of this particular plastic by either the forces of deep-ocean environment or by microbial activity or both. This resulted in a loss of flexibility and a reduction in the original dimension of the plastic tube. Of the various types of electrical insulation exposed near the ocean floor, silicone rubber was deteriorated by the nibbling and biting action of some unidentified mud-dwelling marine organisms. Identical materials which were exposed about 8 feet above the sediment were not affected. Coral concrete specimens which were exposed about 10 feet above the sediment were not affected by marine borers. The concrete specimens placed at this height may have been subjected to attack by relatively few borers because it has been found that the greatest borer activity occurs near the sediment layer and starts to decrease above the sediment layer.

The following nonmetallic materials were not affected by marine organisms or by the seawater environment at this particular test site, and these could probably be used for deep-sea application at this site: concrete; electrical cable insulation such as neoprene, butyl rubber, natural rubber, Teflon, polyethylene, FEP, Bakelite, PVC, and nylon; plastic electrical insulating tape; plastic films and sheets such as Saran, polyethylene, and acrylic; nylon nuts and bolts; rubber tubing (vacuum); nylon parachute cords; and cable clamps made of ethyl cellulose. The electrical cables and plastic films listed above were exposed to seawater about 8 feet above the sediment and were not subjected to the activity of microorganisms of the sediment nor to a variety of mud-dwelling animals found inhabiting the bottom sediment.

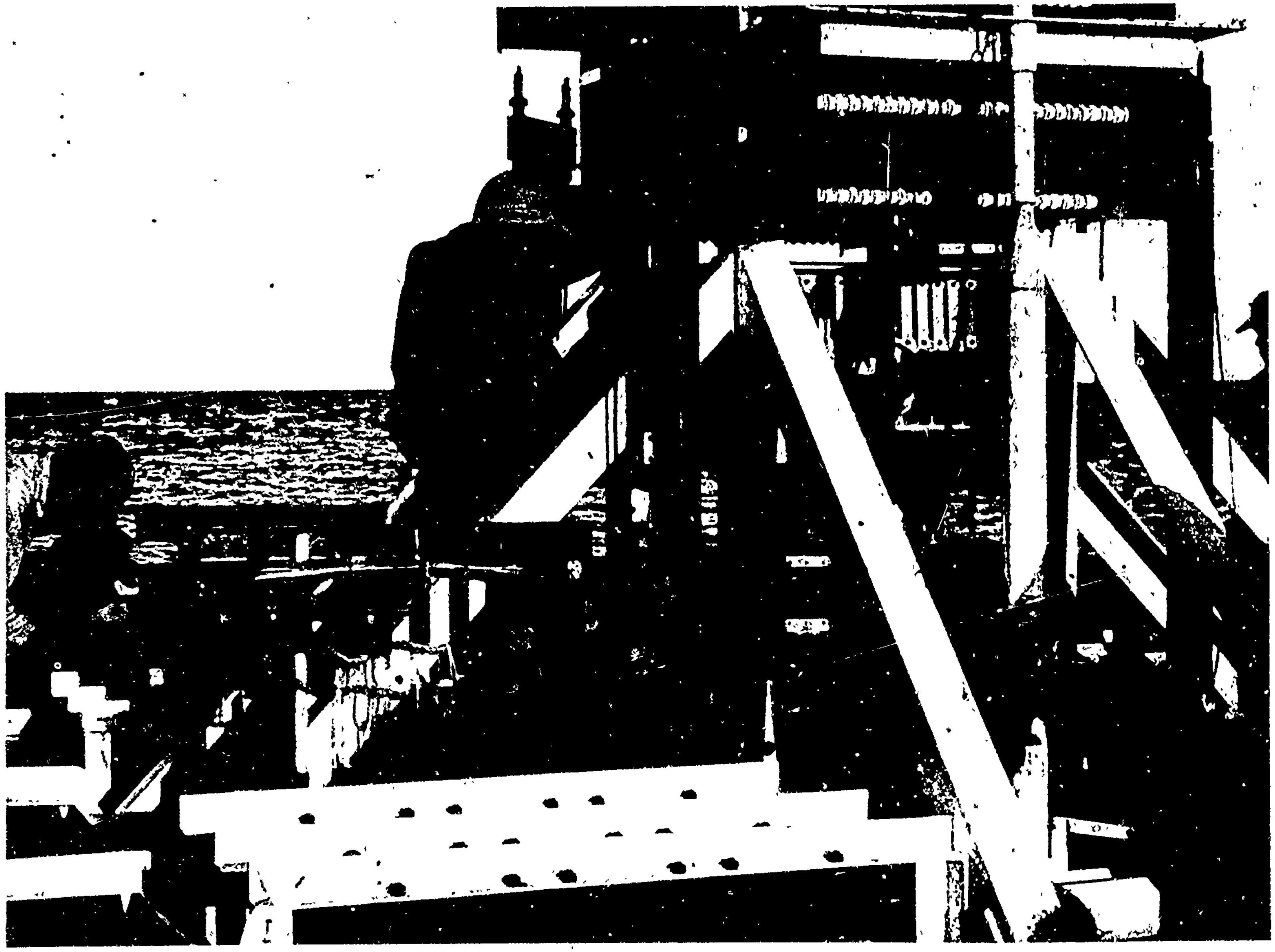


Figure 25. STU I-1 immediately after recovery. Lower section showing test panels and biological test racks still attached to STU frame.

The U. S. Naval Civil Engineering Laboratory made a study of the problem of locating, handling, placing, cooling, and maintaining an unattended nuclear power plant on the floor of the deep ocean.[16] One of the problems considered was whether or not fouling would be encountered at great depths, and whether it would affect the operation of a reactor. From the information obtained about marine growth on the STU I-1 materials, it is envisioned that the finned plates for heat rejection mounted on a reactor unit placed at this particular test site could be completely covered by hydroid growths after prolonged submergence especially over an area of the plates where optimum temperature exists for the attachment and growth of hydroids. In a study[17] to control marine fouling growth in pipe systems by treatment with hot water, water temperatures above 42°C were found to be effective. Deep-sea hydroids and other fouling organisms could probably be discouraged from growing on finned plates if the temperature of the plates was kept above 42°C.

FINDINGS

1. There is considerable biological activity occurring in the sediment at Test Site I.

2. Marine fouling organisms such as bryozoa, tube worms, and glass sponges were found on rock samples collected near Test Site I in 6,000 feet of water.

3. Marine animals such as hydroids, sea anemones, annelid worms, and small white starfish were found on polypropylene and on nylon ropes used in the STU complex.

4. Hydroid growths in trace to heavy amounts were present on all the test specimens placed on the STU. Species of tube worms, limpets, and pectens were the other marine animals found attached to metals, plastics, and on coated test specimens. Typical fouling organisms such as bryozoa and barnacles were not found on these panels.

5. Marine microorganisms were responsible for the deterioration of (a) cotton and Manila rope specimens, (b) jute fibers (burlap coated with coal tar), and (c) vinyl plastic tube (NCEL No. 15).

6. Two species of marine borers were found in wood specimens and have been identified as *Xylophaga washingtona* Bartsch, and *Xylophaga duplicata* Knudsen. The borers were responsible for the deterioration of

   (a) Pine, fir, and greenheart wood. Some of the tunnels made by the borers through pine wood measured about 3/4 inch in diameter. Both of the borer species were found in pine wood; however, only one specie (*Xylophaga duplicata*) was found in greenheart wood,

(b) All of the 3-foot-long plastic rods and tubes. Shallow-to-deep holes were made on the surface of these materials and were restricted to an area where a large wooden bait piece was fitted around each plastic specimen, and

(c) Manila rope specimens. The borers together with microorganisms destroyed the usefulness of the rope. The destruction was complete and rapid by marine borers measuring 1/4 inch in diameter which were found throughout the entire length of the rope specimens. Some of the borers had completely penetrated through the 1/2-inch-diameter rope.

7. Silicone rubber electrical cable insulation was deteriorated by nibbling and biting action of some marine animals.

8. The following materials were not affected by marine organisms: rubber tubing (vacuum), coral concrete, acrylic sheet, Saran and polyethylene films, nylon nuts and bolts, plastic electrical insulating tape, nylon parachute shroud line, ethyl cellulose cable clamps, and various electrical cable insulating materials such as neoprene, butyl rubber, natural rubber, Teflon, polyethylene, FEP, Bakelite, PVC, and nylon.

CONCLUSIONS

1. The materials listed under Finding No. 8 are considered to be suitable for deep-ocean use in an environment such as at Test Site I.

2. Materials such as pine and greenheart wood, Manila and cotton ropes, silicone rubber insulation, friction tape, and jute fibers are susceptible to total biological destruction and are not suitable for deep-ocean use.

FUTURE PLANS

Investigation of the effects of the deep-ocean environment upon materials is continuing.

Test Site I (Nominal Depth of 6,000 Feet)

STU I-4 which had been exposed at this test site since June 1964 at a depth of 6,800 feet was recovered in July 1965. The materials have been examined for biodeterioration and writing of a report has been initiated. STU I-2 which had been exposed at a depth of 5,640 feet since October 1963 was retrieved in October 1965. The materials are presently being examined for biodeterioration.

Test Site II (Nominal Depth of 2,500 Feet)

STU II-2 was emplaced in April 1965. Plans are to retrieve it after a year's exposure at this depth.

ACKNOWLEDGMENTS

Dr. Ruth D. Turner, Harvard University, identified the marine borer specimens. Dr. Raymond B. Manning, Smithsonian Institution, identified the crustacean specimen.

APPENDIX. BIOLOGICAL EFFECTS ON MATERIALS ASSEMBLED ON BIO-RACKS

Materials	NCEL No.	Size and Description	Summary of Results	Remarks
Plastic Rods		3-foot-long specimens commercially available through catalogs.	Borer hole dimensions include two perpendicular measurements of the diameter, and depth.	Numerous holes were made by marine borers on the surface of all the plastic rods in an area where a large wooden bait piece was fitted around each material. The borers had attacked the wood first, and then the surface of the plastic. The damage to the plastic ceased when the wood was totally destroyed.
Cast Acrylic	4	1-inch diam. Basic material is methyl methacrylate.	Over 45 borer holes — the largest measured about 1/8 x 1/8 x 1/64 inch. Heavy hydroid growth.	Other areas of the rod were not affected by borers.
Polystyrene	5	1-inch diam	Over 90 borer holes — the largest measured about 5/16 x 3/16 x 1/16 inch. Heavy hydroid growth.	Other areas of the rod were not affected by borers.
Extruded acrylic	6	1-inch diam	Over 70 borer holes — the largest measured about 1/8 x 1/8 x 1/64 inch. Heavy hydroid growth.	Other areas of the rod were not affected by borers. Species of limpets were attached to the plastic exposed near the sediment.
Polyethylene	7	1-inch diam. Alathon 10.	Over 25 borer holes — the largest measured about 1/8 x 1/8 x 1/64 inch. Light hydroid growth.	A 1-inch-long worm tube made of calcium carbonate was attached to the plastic. Other areas were not affected by borers.
Teflon	10	1/2-inch diam. Polymer used is tetrafluorethylene.	Over 40 borer holes — the largest measured about 1/4 x 1/4 x 1/32 inch (Figure 26). Light hydroid growth.	Other areas were not affected by borers.
Polycarbonate	11	1/2-inch diam	Over 50 borer holes — the largest measured about 1/16 x 1/16 x 1/64 inch. Moderate hydroid growth.	Other areas were not affected by borers.
Cellulose acetate	8	1-inch diam. Light purple, MH flow, extrusion grade.	Over 100 borer holes — the largest measured about 1/4 x 5/16 x 1/16 inch (Figure 27). Heavy hydroid growth.	This particular plastic absorbed a considerable amount of water. As a result, the plastic rod which was placed in a bio-rack with very little end clearance was bent like a bow when recovered from the sea. Other areas were not affected by borers.
Phenolic laminate	12	3/4-inch diam. Grade 911, purified woven canvas base, molded rod, impregnated with phenolic resin, MIL-P-18324. Used for underwater applications.	Over 30 borer holes — the largest measured about 1/8 x 3/16 x 1/64 inch. Heavy hydroid growth.	Other areas were not affected by borers.

Continued

Materials	NCEL No.	Size and Description	Summary of Results	Remarks
Nylon	13	3/4-inch diam	Over 40 borer holes — the largest measured about 5/16 x 1/4 x 1/16 inch. Heavy hydroid growth.	Other areas were not affected by borers.
Delrin	14	3/4-inch diam	Over 35 borer holes — the largest measured about 1/8 x 5/16 x 1/32 inch. Heavy hydroid growth.	Other areas were not affected by borers.
Plastic Tubes		3-foot long, 1-inch OD, 1/8-inch-thick wall. Commercially available.		See remark under Plastic Rods above.
Vinyl	1	Semirigid general-utility chemical hose, black.	Over 140 borer holes — the largest measured about 1/4 x 1/4 x 1/16 inch (Figure 28). Heavy hydroid growth.	Several borer holes about 1/64 inch deep and 1/32 inch wide were found in other areas.
Vinyl	2	Flexible general-utility chemical hose, black.	Over 130 borer holes — the largest measured 1/4 x 1/4 x 1/16 inch. Heavy hydroid growth.	Other areas were not affected by borers.
Vinyl	3	Flexible fuel and lubricating tube, yellow.	Over 70 borer holes — the largest measured about 5/16 x 1/4 x 1/16 inch. Heavy hydroid growth.	Other areas were not affected by borers.
Vinyl	15	Flexible low-temperature tube, black.	Over 15 borer holes — the largest measured about 3/16 x 1/4 x 1/64 inch. No hydroid growth. Entire surface covered with heavy bacterial slime growth.	The tube had shrunk about 1/8 inch in diameter and had become quite rigid. As a result, the friction tape wrapping had slipped to the lower end of the tube. This tube seemed to contain some chemicals preferred by marine microorganisms as a source of food. Some particular chemical product used in the formulation may have leached out.
Plastic Pipe				
Polyvinyl chloride	9	Unplasticized, gray. 1-5/16-inch OD.	Over 30 borer holes — the largest measured about 1/16 x 1/16 x 1/16 inch.	Other areas were not affected by borers.
Rope		1/2-inch diam, 4 feet long.		One set of cotton and Manila rope specimens was exposed a few inches above the sediment, while the other set of ropes was exposed about 3 feet above the sediment.
Cotton Cotton	A1 118		Deteriorated by marine microorganisms.	The recovered cotton rope was in such a deteriorated condition that it started to crumble into small pieces when handled. Marine borers were not involved in the destruction of the rope (Figure 29).

Continued

Materials	NCEL No.	Size and Description	Summary of Results	Remarks
Manila Manila	A2 A19		Deteriorated by marine microorganisms as well as marine borers.	A tensile-strength test could not be performed because the rope was severely deteriorated. Numerous 1/4-inch-diameter borers were found throughout the rope. Some had penetrated completely through the rope (Figure 30).
Electrical Conductors		0.015-inch-thick insulation over No. 16 tin-coated copper wires, each 12 inches long. Ends sealed with silicone rubber cement.		See Table VI for results on insulation resistance and voltage breakdown tests.
Polyethylene Polyvinyl chloride GR-S (SBR) rubber Neoprene rubber Silicone rubber		See Table VII.	No sign of animals nibbling on insulation.	The materials were exposed about 8 feet above the sediment in water where fewer bacteria are present than in sediment.
Electrical Conductors (Same material as above)		All 10 feet long.	Silicone rubber insulation deteriorated by nibbling and chewing action by some marine animals. Voltage breakdown test revealed several areas where the wire was exposed to seawater. Other materials were not affected.	These were exposed a few inches to 2 feet above the sediment and were accessible to mud dwelling animals.
Electrical Cables		10-inch-long single and multiconductor cables. Ends sealed with silicone rubber cement. OD before exposure:	Not affected. OD after exposure:	Cable materials were assembled in special racks aboard the STU and exposed about 8 feet above the sediment. Insulating materials were examined under a stereoscopic microscope for signs of cracking and borer damage (Figure 31).
Butyl jacket	65, 69 90, 94	0.508-inch, black.	0.509-inch. Hydroid growth present.	Four materials of each. The OD measurements were taken after 4 months storage at ambient room temperature.
Neoprene jacket	73, 75 98, 100	0.491-inch, black.	0.488-inch. Hydroid growth present.	
Natural rubber	87, 89 112, 113	0.356-inch, black.	0.350-inch. Hydroid growth present.	
Silicone rubber	83, 84 108, 109	0.324-inch, blue.	0.321-inch. Hydroid growth present.	The impression of the twisted conductors, produced by hydrostatic pressure, was visible on the insulating material.
Teflon (TFE)	66, 68 91, 93	0.080-inch, white with black stripe.	0.080-inch. Trace of hydroid growth present.	Four materials of each. The OD measurements were taken after 4 months storage at ambient room temperature.

Continued

Materials	NCEL No.	Size and Description	Summary of Results	Remarks
Fluorinated ethylene propylene (FEP)	70, 72 95, 97	0.120-inch, clear.	0.118-inch. Trace of hydroid growth present.	
Nylon "33Z"	79, 80 104, 105	Clear, transparent. Jacketed over tinned copper shield.	Irregular surface. Hydroid growth present.	
Bakelite "9033"	81, 82 106, 107	0.243-inch, black.	0.247-inch. Hydroid growth present.	The impression of the twisted conductors, produced by hydrostatic pressure was visible on the insulating material.
Nylon	74, 76 99, 101	0.155-inch, white (opaque).	0.155-inch. Hydroid growth present.	
Polyvinyl chloride (PVC), Geon 8800	85, 86 110, 111	0.323-inch, black.	0.315-inch. Hydroid growth present.	See remark on NCEL No. 81, 82, 106, 107.
Polyethylene	77, 78 102, 103	0.199-inch, translucent, low density "2005."	0.201-inch. Hydroid growth present.	
Wood				
Douglas fir		Two 2 x 4 x 30-inch pieces bolted together.	Completely destroyed by marine borers. The wood pieces were not recovered.	Wooden bait pieces over plastic rods and tubes.
Pine		Two 3/4 x 3 x 24-inch panels.	Riddled by marine borers.	This particular wooden panel was recovered because it was sandwiched between two 3 x 24-inch metal sheets about 6 feet above the sediment. Some of the tunnels made by the borers through the board measured about 3/4 inch in diameter (Figures 32 and 33). Since this was the thickest board recovered, it is not known whether the borers had reached their maximum growth. Two species of borers were found in the wood. These have been identified as <u>Xylophaga washingtona</u> and <u>Xylophaga duplicata</u>. Fruiting bodies of a fungus belonging to the Ascomycetes have been found as well as evidence of attack possibly by marine bacteria on the surface of the specimens (walls of the wood fibers). Further study of the fungus and bacteria will be conducted on wood panels recovered from the deep ocean.
(a) Greenheart		1/4 x 3 x 24-inch panel.	Not affected. Only a few small borer holes on the surface of wood.	The wood was exposed deeply into the sediment. A thick layer of bottom mud had to be scraped off both sides of the wood panel before being examined.

Continued

Materials	NCEL No.	Size and Description	Summary of Results	Remarks
(b) Greenheart		1/4 x 3 x 24-inch panel.	Riddled by marine borers.	The wood was exposed a few feet above the bottom sediment. The majority of the borers inside the wood measured about 1/4 inch in diameter. A single specie, Xylophaga duplicata Knudsen, was found in greenheart (Figures 34 and 35).
Jute Fiber				
Burlap		5 by about 8 inches	Completely deteriorated by marine microorganisms. Sections of burlap coated with coal tar were also deteriorated by the activities of microorganisms. Heavy hydroid growth.	Several layers of burlap were wrapped around each of the plastic rods and tubes. The first layer was coated with coal tar to secure it to the plastic material. A few immature borers were found in the jute material.
Tape				
Plastic tape, electrical		0.010 x 3/4-inch, pressure-sensitive, adhesive, insulation tape, black.	Not affected. Heavy hydroid growth over tape.	Wrapped around plastic rods and tubes. The tape was in good condition. It had excellent adhesive quality remaining when unwrapped, and it probably could be reused.
Friction tape		0.010 x 3/4-inch, black.	Deteriorated by microbial action and by seawater.	Wrapped around plastic rods and tubes. The fiber material in the tape was deteriorated so that the tape could not be unwrapped in one piece. The tape material could only be removed in very small pieces. Only a few immature borers were found.
Plastics			Not affected.	
Acrylic sheet		5/16 x 4 x 13-inch, transparent (clear).	Moderate hydroid growth. Two worm tubes attached to the surface.	The material was used to hold the 10-inch-long single and multiconductor electrical cables. The plastic sheet was not warped. Could be reused.
Nylon cord		Nylon parachute shroud line, white.	Moderate hydroid growth.	The cord was used to secure the cotton and Manila ropes to the wooden bait piece and to the plastic rods and tubes. Could be reused.
Cable clamps		1/16 x 3/8-inch clamps for 5/8-inch cables. Ethyl cellulose, yellow.	Moderate hydroid growth.	Could be reused.
Polyethylene film		0.004 x 3 x 18-inch, two of each.	Slight hydroid growth.	The 18-inch-long film was made into a roll and held in place with a plastic cable clamp. The material was exposed about 8 feet above the sediment. Recovered material remained flexible and could probably be reused.
Saran film		0.002 x 3 x 18-inch, two of each. Vinylidene chloride.	Slight hydroid growth.	Same as above.

Continued

Materials	NCEL No.	Size and Description	Summary of Results	Remarks
Nylon nuts and bolts		1/4-inch diam		Could be reused.
Rubber tubing		5/8-inch OD, 3/16-inch wall thickness, 1/4-inch-diam hole, red. Used for vacuum or pressure.	Not affected. Heavy hydroid growth over entire tube.	The rubber material was in excellent condition. Surface crackings were absent. Could be reused.
Plastic Film and Sheets				The plastic films and sheets were nailed to the wooden bait piece fitted over plastic rods and tubes. These materials were lost when the wooden bait piece was destroyed by borer and was not recovered.
Saran film	A10	0.002 x 3 x 18-inch, light brown.		
Teflon film		0.004 x 3 x 18-inch, white.		
Cellulose acetate	A12	0.015 x 3 x 18-inch, clear.		
Cellulose acetate butyrate sheet	A13	0.020 x 3 x 18-inch, clear.		
Styrene sheet, high-impact	A14	0.020 x 3 x 18-inch, white.		
Coral concrete	A16a A16b A16c A16d	6.2 x 3-inch cylinder. Made of Guam reef coral and seawater.	Not affected. Light hydroid growth.	Borers did not attack the concrete, which was exposed about 10 feet above the sediment. The average compressive strength of the four cylinders was 1,740 psi. The average compressive strength of four control specimens (left in a 100% humidity room for 3 years) was 2,280 psi (Figure 36).

Table VI. Deep-Ocean Effects on Insulation Resistance of Electrical Insulating Materials

Materials (15 mils thick)	Insulation Resistance (meg)		Voltage Breakdown3/
	Before Exposure1/	After Exposure2/	
Polyethylene	20,100,000	2,500,000	None
Polyvinyl chloride	4,400,000	500,000	None
Silicone rubber	6,200,000	850,000	None
GR-S rubber (SBR)	5,500,000	650,000	None
Neoprene rubber	36,000	16,000	None

1/ Average of 8 wires
2/ Average of 4 wires
3/ Tested at 1,000 volts AC for 10 seconds

Table VII. Materials Used in the Formulation of Insulating Materials (15 mils thick)

Test Specimens	Plasticizer	Fillers	Antioxidant
Polyethylene (Standard polyethylene insulation)			
Polyvinyl chloride (PVC)			
GR-S rubber (SBR)	Cumarone-indene resin and microcrystalline wax	Hard clay and water-ground whiting	Polymerized trimethyl dihydroquinoline
Silicone rubber			
Neoprene (Type W)	Light process oil and petroleum	Hard clay	4, 4 thiobis (6-tert-butyl m-creosol)

Figure 26. Holes made by borers on the surface of a Teflon plastic rod around an area under the wooden bait piece. The wood was destroyed by the borers.

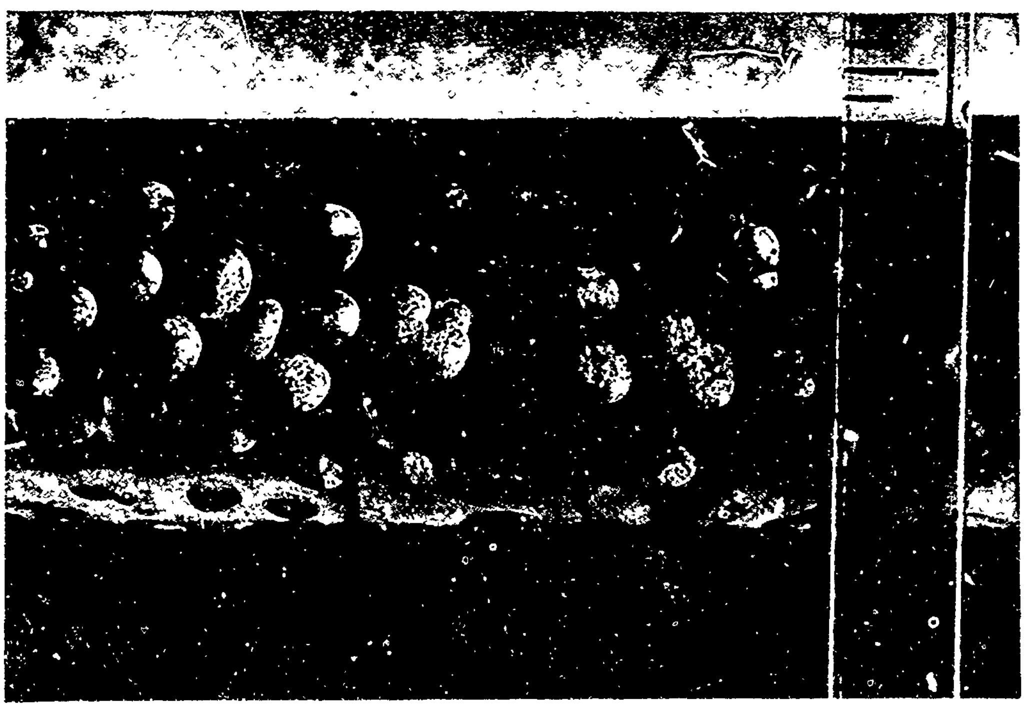


Figure 27. Over 100 holes made by borers on the surface of a cellulose acetate plastic rod around an area under the wooden bait piece. The wood was destroyed by the borers.

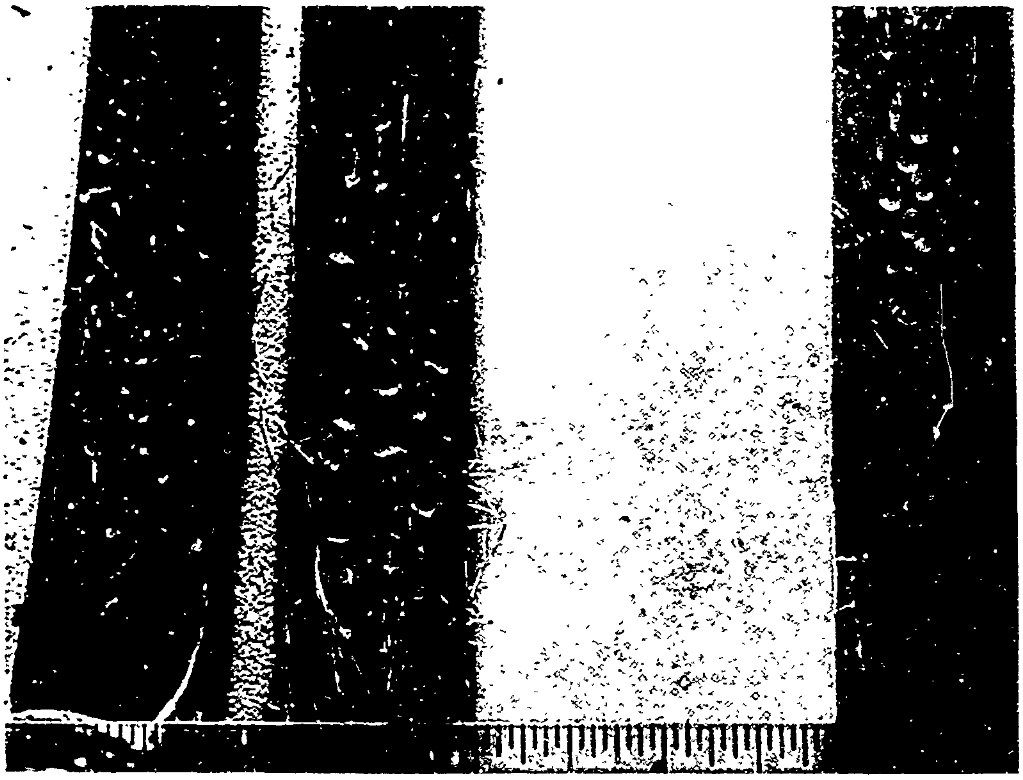


Figure 28. Holes made by borers on surface of vinyl plastic tubes around an area under the wooden bait piece. The wood was destroyed by the borers.

Figure 29. The 1/2-inch-diameter cotton ropes deteriorated by microorganisms. The rope fibers crumbled when handled.

Figure 30. The 1/2-inch-diameter Manila rope deteriorated by the combined effects of microorganisms and borers. Two borers are visible.

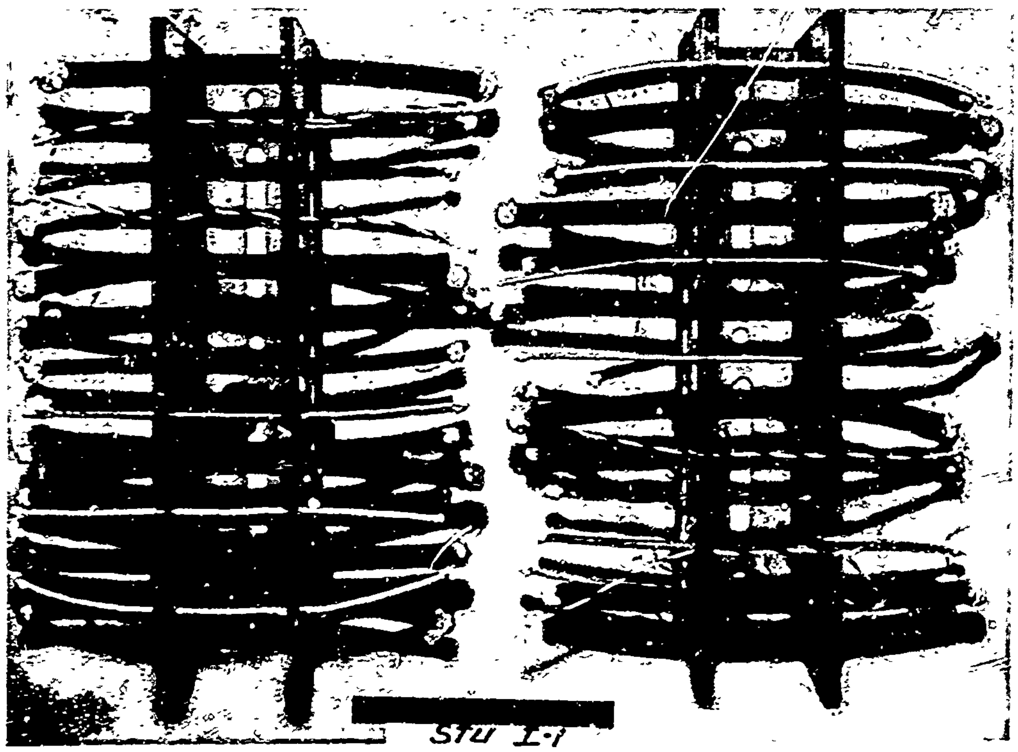


Figure 31. Single and multiconductor electrical cables after 35 months near the sea floor. Hydroid growth can be seen attached to the insulation.

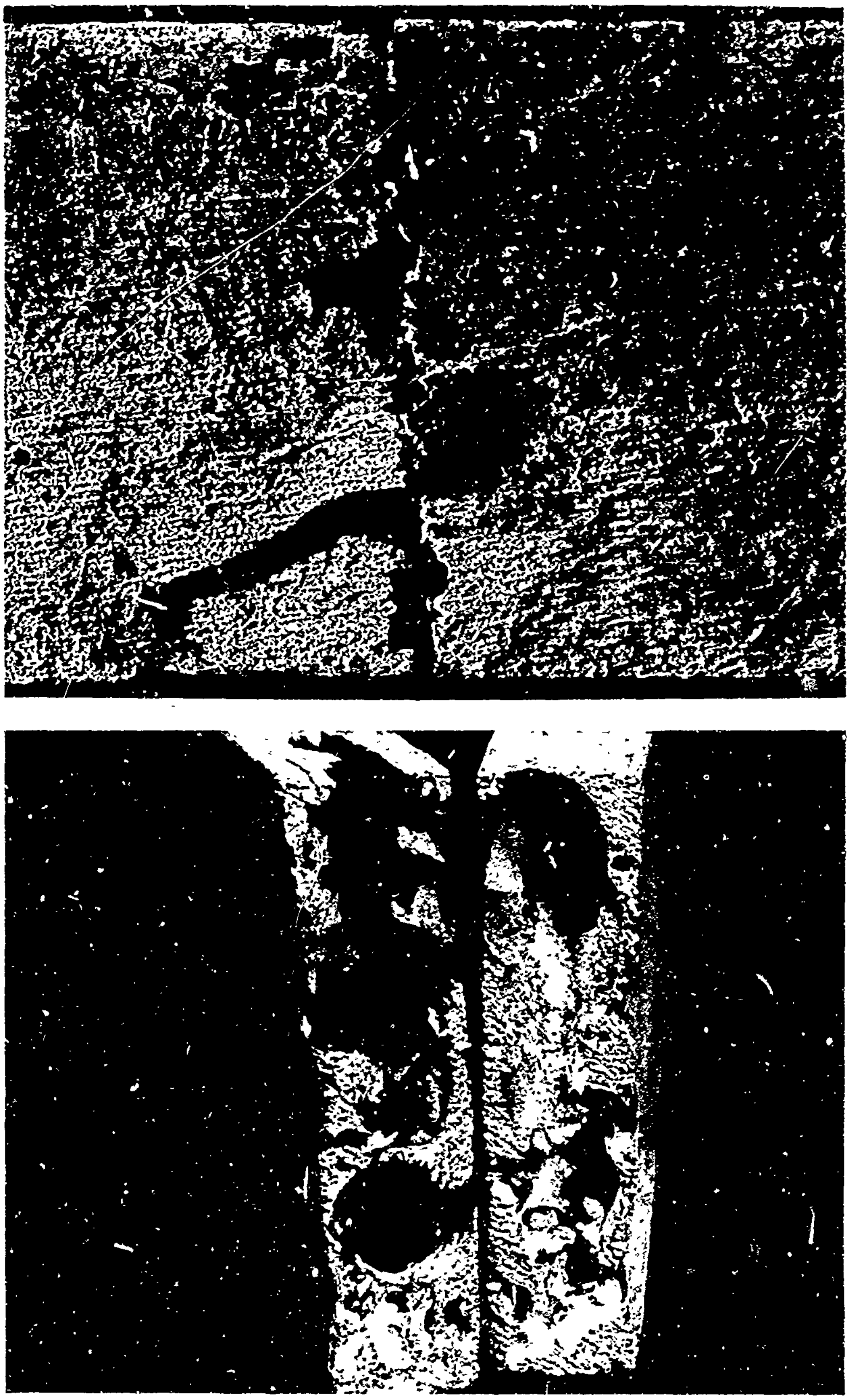


Figure 32. Pine-wood panel riddled by borers. Top panel shows numerous small entrance and breathing holes. Bottom panel shows the interior of same wood.

Figure 33. Pine-wood panel riddled by borers. The panels were sandwiched between two metals.

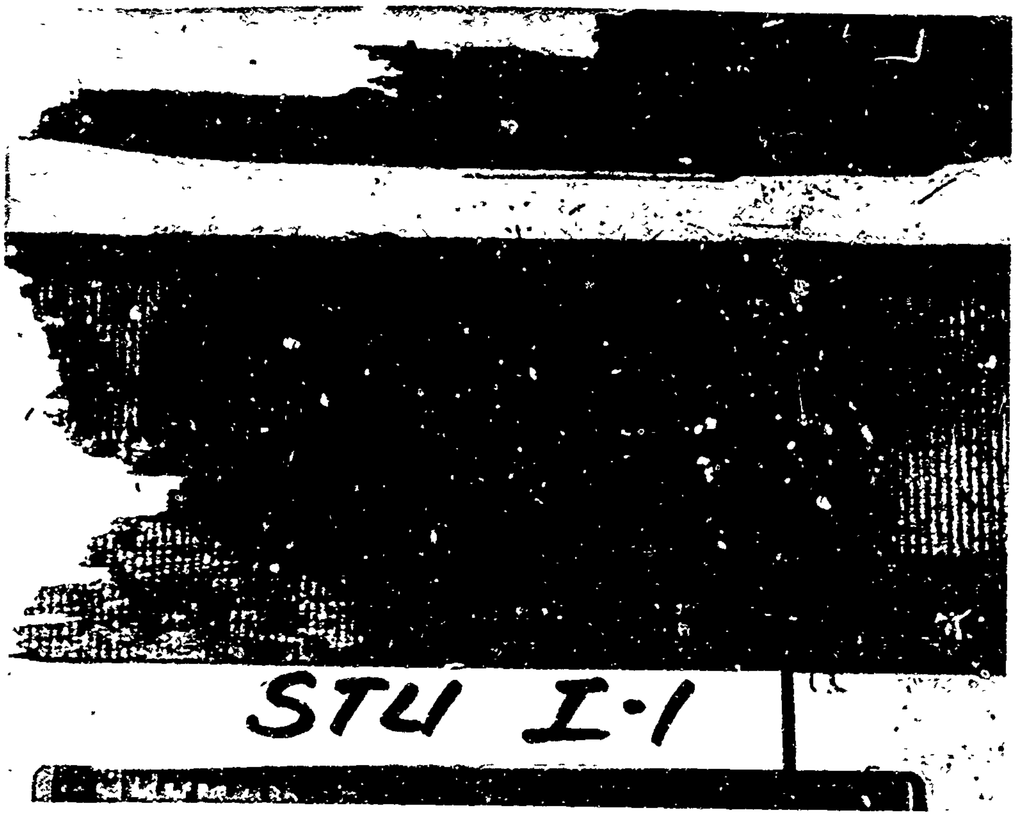


Figure 34. Greenheart wood riddled by borers, Xylophaga duplicata Knudsen.

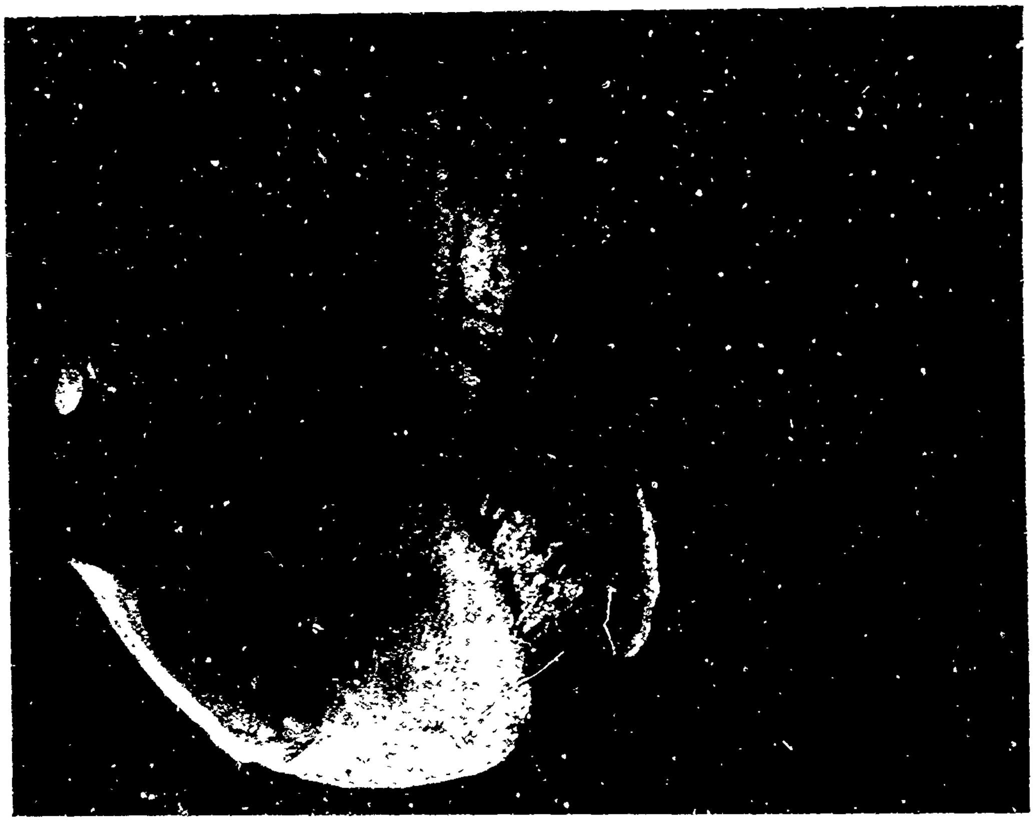


Figure 35. Xylophaga duplicata Knudsen. The 1/4-inch-diameter borer was found inside a greenheart wood panel.

Figure 36. Cylindrical coral concrete specimens exposed about 10 feet above the bottom sediment for 35 months.

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This is Part III of a series of reports on the biological deterioration of materials in the deep ocean. It covers the data obtained after exposing 1,318 test specimens of 316 different materials for 35 months on the Pacific Ocean floor at a depth of 5,300 feet (Test Site I). The materials were attached to a Submersible Test Unit (STU). The STU was retrieved in February 1965 and returned to the Laboratory for tests and analyses.

Hydroid growths were found on all the test specimens placed on the STU. A few species of tube worms were found attached to metals, plastics, and coated test specimens. Most of the plastics and all the rope materials were covered with bacterial slime growth. Cotton and Manila rope specimens were severely deteriorated by bacterial action. Wood panels, plastics, and Manila ropes were attacked by marine borers. Metals, natural and butyl rubber, and certain plastic materials were not affected.

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